THE ROLE OF THE HEPATIC ARTERY IN LIVER HEMODYNAMICS: QUANTITATION AND SUGGESTED MECHANISM

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ABSTRACT

The hemodynamics of the liver with its dual blood supply is unique. The liver depends entirely on the hepatic artery to regulate total hepatic flow since the portal vein is unable to control inflow to the liver. The existence of stable hepatic blood flow is essential for the maintenance of hepatic functions. The reservoir (capacitance) function, the clearance of many compounds (such as hormones) and the stability of intrahepatic sinusoidal pressure depends on hepatic blood flow.

It has been long observed that the hepatic arterial flow responds in a reciprocal fashion to changes in portal flow in order to maintain total hepatic flow steady, an observation referred to as the hepatic arterial buffer response. On the other hand, the occurrence of changes in hepatic arterial resistance in response to changes in arterial perfusion pressure (i.e. autoregulation) remains controversial. The mechanisms underlying both responses have not been fully elucidated, although a myogenic mechanism has been suggested.

This research study was designed first, to quantitate the hepatic arterial buffer response and the hepatic arterial autoregulation and second, to examine the likely mechanisms governing these responses. An anesthetized cat model with intact liver and intact blood supply was used. The hepatic arterial autoregulation response and the buffer response were induced by the use of a micrometer screw-clamp placed around

the hepatic artery and the superior mesenteric artery. Blood flow in both arteries was measured by electromagnetic flow probes.

The results indicated that the buffer capacity is variable. The buffering efficiency increases with the decrease in portal flow to reach a maximum value of 24% at 60% decrease in portal flow. Although further decreases in portal flow were accompanied by progressive increases in hepatic arterial conductance, the buffering efficiency declined. Our results showed the existence of weak hepatic arterial autoregulation. Both the buffer response and autoregulation were found to be mediated through adenosine. A unified hypothesis has been suggested according to which adenosine is secreted by specialized cells in the space of Mall close to the resistance arterioles of the hepatic artery. This intrinsic dilator can act on the resistance arterioles or be washed away by portal or hepatic arterial flow. Reduction in either flow would result in accumulation of adenosine resulting in vasodilation of the hepatic artery. An increase in either flow would result in vasoconstriction. Our data support the dilator washout theory.

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1. INTRODUCTION

1.1 GENERAL CONSIDERATIONS

The liver is the largest single internal organ in the body, weighing 1200 to 1800g in the average adult. It is located under the diaphragm and occupies most of the right hypochondrium and part of the epigastrium of the abdomen. The liver is covered completely by a dense connective tissue layer (Glisson's capsule) that lies beneath the peritoneum.

Blood is supplied to the liver by two routes: the hepatic artery with well oxygenated blood, and the portal vein which drains less oxygenated blood from the splanchnic organs and the spleen. Portal blood contains nutrients absorbed from the intestinal tract, hormones released from some splanchnic organs (eg. pancreas) and many other biologically active and inactive products. The liver is interposed between the gastrointestinal tract and the general circulation. Thus, under normal conditions, all substances absorbed from the gut and released into the portal blood stream must flow through the liver before entering the systemic circulation and can be subjected to presystemic treatment and/or elimination.

Unlike other organs, the liver has more than one major function. As a part of the digestive system, the liver synthesizes bile and bile salts to facilitate fat absorption. It stores, synthesizes and metabolizes nutrient materials. The liver has a major excretory function; it clears the circulation of a wide variety of compounds, some of which need to be converted to less toxic (detoxification) or to

hydrophilic compounds before excretion. Another major function of the liver is its hemodynamic role in the systemic circulation. The hepatic blood vessels contain about 14% of the total blood volume of the body (Greenway and Lister, 1974). The liver contracts in response to hypovolemia, as during hemorrhage, expelling a volume of blood equal to 26% of the blood volume loss, regardless of the extent of that loss (Lautt et al., 1980). The hepatic artery contributes significantly to the hemodynamic function of the liver as will be discussed later. Other important functions of the liver include, in brief, blood cell formation (during infantile life), plasma proteins synthesis (such as albumin, prothrombin, etc.) and synthesis of heparin. Liver synthesizes the clotting factors and shares in the destruction of aged red blood cells. The liver is a storage depository for Vitamin A, D and B_{12} and for iron. The liver is the largest store for glucose (as glycogen). The liver acts as a sensory organ as well. It has receptors for glucose, sodium, colloid, fatty acids (free long-chain ones), temperature and pressure. The liver acts as an endocrine gland that controls blood volume through the secretion of two hormones, somatomedin and natriuretic hormone, which control sodium salt excretion from the kidney. The liver is the site of conversion of some hormones from one form to another, such as the conversion of some steroid hormones to testosterone, especially in females. Along with the muscles, the liver

contributes to thermoregulation since it produces heat which is adjustable to the body requirement. The liver is responsible for the formation and excretion of urea. Lastly, the liver acts as a buffer for the glucose level in the plasma to maintain stable blood sugar.

This work will deal exclusively with the hemodynamic function of the liver and the significance of the hepatic arterial contribution to this major function.

To comprehend the hemodynamic function of the liver, a proper understanding of the hepatic circulation and its unique microvascular anatomy is required.

1.2 THE MICROVASCULAR ANATOMY AND FUNCTION

Inside the liver the hepatic artery and portal vein break up into countless numbers of arterioles and terminal portal venules. The terminal hepatic arterioles are much smaller in diameter compared to the terminal portal venules which make them more resistant to the blood flow. The circulation across the hepatic sinusoids occurs at a low pressure gradient. The parallel subdivisions of the hepatic artery and portal vein are accompanied at their terminal levels by bile ductules, lymph vessels, and nerves which originate from similar preterminal structures in a small triangular portal tract (Rappaport et al., 1954). Thus, the portal tract is a channel which contains the terminal portal venules, the hepatic arterioles and the other terminal structures mentioned above,

separated from the sinusoidal parenchymal cells by a single layer cell wall called the limiting plate. The space between the limiting plate and the microvessels within the portal tract is called the space of Mall (Figure 1). Little is known about the function and structure of the cells that constitute the limiting plate and about the space of Mall in particular.

Both the terminal portal venules and hepatic arterioles end in the sinusoids and all afferent blood has to flow through them before reaching the efferent vessels, the terminal hepatic venules. Hence, liver sinusoids are synonymous with capillaries in other vascular beds. In human and hamster livers, all arterioles terminate directly into sinusoids adjacent to the portal tract and no intralobular arterioles exist (Yamamoto et al., 1985). Rat liver is somewhat unique. It features numerous arterioportal venous anastomoses and only some arterioles open directly into liver sinusoids. Moreover, hepatic arterioles further subdivide in the portal tract to make a unique plexus around the bile ductules before their termination into portal venules or sinusoids, a phenomenon apparently not present in human liver (Yamamoto et al., 1985). Therefore, care should be taken in deriving conclusions from studies on hepatic circulation performed on rat liver.

The sinusoids - the specialized wide-pore capillaries of the liver - are the space between the endothelial lining of the one-cell thick hepatic plates, which form a complex and

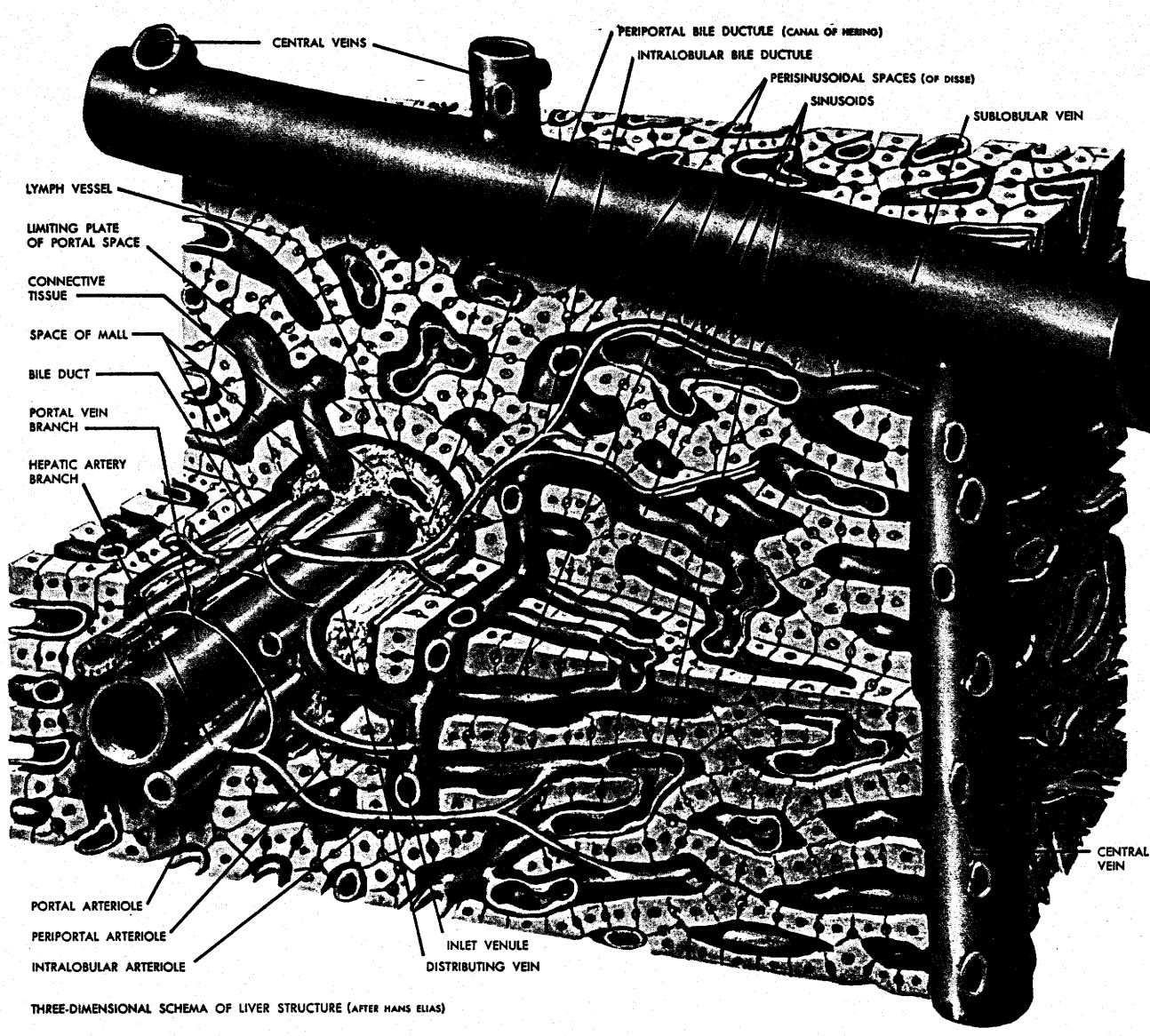

Fig. 1 Three-dimensional schema of liver structure. Note that the space of Mall is referred to in some books as the periportal space. The artist speculated the presence of intralobular arterioles. The existance of such arterioles have not been confirmed histologically. (The CIBA Collection of Medical Illustrations, Vol. III, The Digestive System, Part III, Section XV, Plate 6.)

richly anastomosing labyrinth-like system (Motta et al., 1978). The sinusoids empty into the terminal hepatic venules, from which they debouch into the tributaries of the hepatic venous system. The hepatic veins, thence empty into the inferior vena cava. The sinusoids are lined mainly by two types of cells; the endothelial cells and Kupffer cells, which are reticuloendothelial cells capable of phagocytizing bacteria and other foreign bodies in the blood. The endothelial cells of the sinusoids are a unique type of endothelial lining consisting of cell bodies with flattened processes perforated by small fenestrae of about 100nm in size (range 10 to 200nm in diameter). These fenestrae are arranged in groups and often called sieve plates (Wisse, 1970). The endothelial lining of the sinusoids is separated from the parenchymal hepatic plates by a narrow space called the space of Disse. Therefore, the space of Disse is bordered, at one side, by a thin endothelial layer and, at the other side, by the plasma membrane with its microvilli of the parenchymal cells. The total space between each two opposing hepatic plates (i.e. the sinusoidal space + 2 spaces of Disse) is called the hepatic lacuna. The sinusoidal endothelial lining lacks a basal lamina and for that reason the fenestrae constitute an open connection between the sinusoidal lumen and the space of Disse. It is believed that the major part of the transport and exchange of fluid, solutes and particles between the blood and the space of Disse takes place through these

open sieve plates. The space of Disse as such is a unique form of interstitial space filled with fluid similar to that of plasma since it is freely accessible to all the large molecules contained in the plasma. Red cells are larger than the fenestrae and do not have access to the space of Disse. Therefore, in the liver, the main barrier between the plasma and the interior of the hepatocytes is the parenchymal plasma membrane. The above description brings us to the conclusion that the sinusoids have a unique structure that allows maximum contact between the hepatocytes and the blood perfusing the liver. The distribution of endothelial fenestration populations is not static. The diameter of the fenestrae can increase or decrease in response to many physiological and pathological situations such as hypoxia, blood pressure, irradiation, endotoxemia, alcoholic intoxication, etc. (Wisse et al., 1985). Apart from the dynamics of fenestrae diameter, the kinetics of fenestrae number (fenestrae per square micron) has been reported as well. Alcohol administration increases the size, but decreases the number of endothelial fenestrae (Mak and Lieber, 1984). Normally, the diameter of fenestrae decreases slightly as we move from sinusoids adjacent to the portal tract (or triangle), i.e. zone 1, to sinusoids adjacent to the terminal hepatic venules, i.e. zone 3, whereas the frequency increases, resulting in an increase in porosity (Wisse et al., 1985). These gradients in the frequency and diameter of fenestrae are paralleled by gradients in the

morphometry of sinusoids. Sinusoids in zone 1 are narrow and more tortuous than those of zone 3 (5.9uM in zone 1 and 7.1uM in zone 3 measured from in vivo microscopic preparation) (Wisse et al., 1985). These findings indicate that both red blood cells (7.32uM in diameter) and white blood cells (mean of 8.5uM in diameter) are bigger than inlet sinusoids of zone 1. This implies deformation of the cells during passage through sinusoids from zone 1 to zone 3, which was observed by using in vivo microscopy (McCuskey et al., 1979). Red blood cells pass in a single row and adapt their shape to size differences and curvitures. White blood cells are less plastic and do not adapt easily. They interrupt blood flow and are either pushed out after mild adaptation or attach to the endothelium and migrate by active movement. This peculiar pattern of movement of blood cells through liver sinusoids leads to two important hemodynamic observations. First, blood cells (the white ones in particular) press and squeeze the space of Disse as they move and massage the endothelial lining, forcing the fluid content of the space of Disse to flush out through the fenestrae to the sinusoidal lumen compartment. This fluid movement will contribute to the vital process of hepatic uptake and exchange. Second, as a result of blood cell movement, fluid in the space of Disse along with the plasma column in the sinusoids is pushed downstream toward terminal hepatic venules. Since all of the exit points are adjacent and flow is downstream, flow in adjacent sinusoids is

concurrent. In other words, blood flows in one direction from the inlet sinusoids in zone 1 to zone 3 and there is no chance for any biological product released from the liver downstream to diffuse upstream where the resistance vessels controlling blood flow are located. This point will be further discussed later.

The functional hepatic microvascular circulatory unit is the simple liver acinus (Rappaport, 1973). The acinus is a small parenchymal mass or glomus, irregular in size and shape, arranged around an axis consisting of a terminal hepatic arteriole, terminal portal venule, bile ductule(s), lymph vessels, and nerves (Figure 2). An acinus lies between two or more terminal hepatic venules, the so-called central veins, with which its vascular and biliary axial channels interdigitate. The interdigitation of terminal branches originating from three or more triangular portal spaces around one terminal hepatic venule may create a vascular pattern simulating a hexagon which is the classical histological appearance of liver sections under the light microscope. Hence, the difference between the classical liver lobule and liver acinus is that the liver lobule is the mass of parenchymal cells and sinusoids that drain into a terminal hepatic venule (i.e. hepatic central venule) whose cross-section resembles a hexagon histologically, while the acinus is a small mass of parenchymal cells nourished by blood supplied from one triangular portal space or tract (i.e.

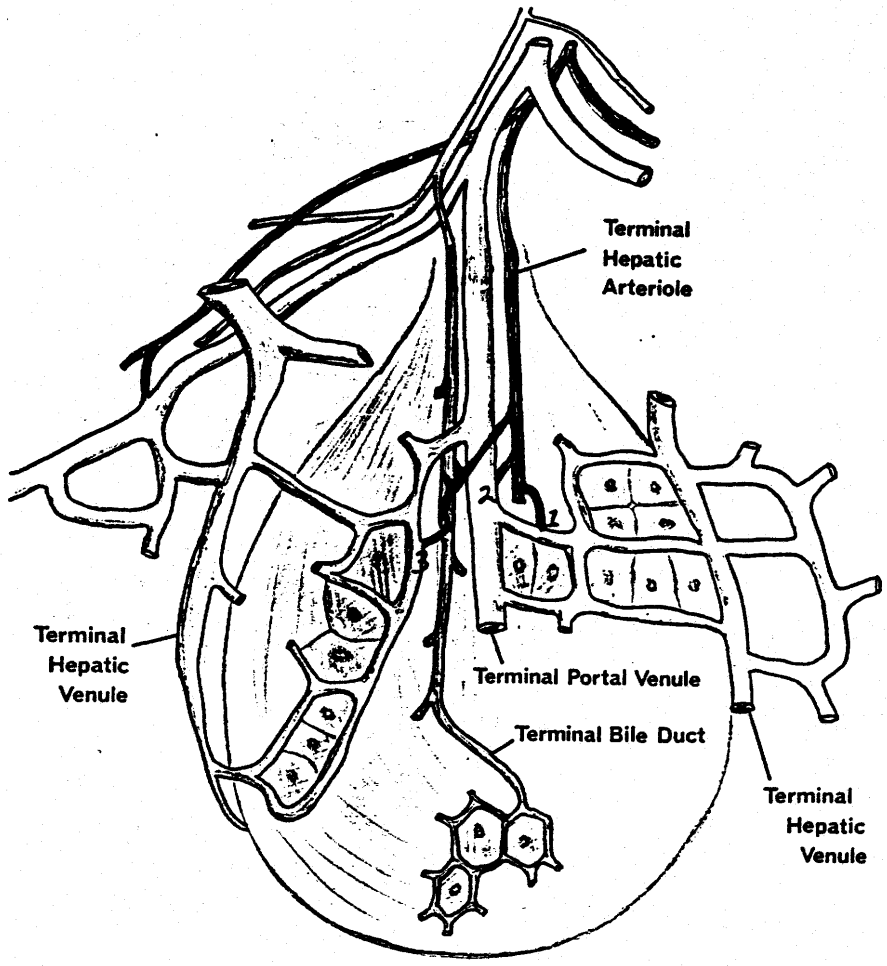

Fig. 2 The liver acinus (microcirculatory hepatic unit). Acini cluster like grapes at the ends of portal and arterial branches. Blood enters the acinus at its centre (zone 1) and flows outward to drain into a terminal venule at its periphery. Level of oxygenation decreases as blood passes from zone 1 to zone 3. (From Lautt, 1977, Gastroenterology 73: 1163-1169.)

portal venule and hepatic arteriole(s)). The liver acinus, and not the hepatic lobule, is considered as the functional unit since it is the smallest parenchymal mass whose function is controlled independently of neighbouring areas. Whereas, there is no structural nor secretory unity in the imaginary hexagonal lobule.

The liver acinus is subdivided, according to O_2 content, into three circulatory zones which surround the axial structure. The periportal microenvironment has the highest O_2 tension derived from the terminal hepatic arteriole and is called zone 1. The perivenular microenvironment is the most distant from the blood supply and receives blood that has already exchanged gases (i.e. low O_2 tension) and metabolites, and is called zone 3. Zone 2 is the transitional zone in between zone 1 and zone 3. It is evident that cells of zone 3 are most sensitive to damage through ischemia, anoxia, congestion, and nutritional deficiency (Rappaport, 1963). The changes in living conditions between cells in zone 1 and 3, due to arterial irrigation and O_2 gradient that decreases toward zone 3, has been translated as change in the metabolic activities of these zones. Zone 1 enzymatic activity is preferentially geared to synthetic (eg. protein and glycogen) and regenerative processes. Whereas catabolic processes (eg. fat, drugs and pigment) are facilitated and enhanced in zone 3 (Jungermann and Sasse, 1978). Metabolic and enzymatic activity of zones 1-3 should not be considered as having a

static distribution. Necrosis of the parenchymal cells in one zone leads to a shift of the metabolic function to the healthy cells of the neighbouring zone (Sasse, 1975).

1.3 HEPATIC VASCULATURE

The interposition of the liver between the gastro-intestinal tract and the general circulation, makes the liver obliged to receive all the blood outflow from the gastro-intestinal tract and the spleen. In other words, the liver is unable to control its portal blood inflow which can only be regulated through blood vessels feeding the gastrointestinal tract and the spleen (i.e. superior and inferior mesenteric arteries, splenic artery, celiac artery, etc.). Therefore, the only way for the liver to control or regulate its total blood flow is through the hepatic artery. The hepatic artery is a dynamic vessel and capable of such regulation. Hepatic arterioles and terminal hepatic arterioles have muscular walls and sphincters which enable the arterioles to regulate flow, pressure, oxygen content and tension in the microcirculatory unit they supply (Rappaport, 1981).

The presence of unmyelinated nerve fibres along with the smooth muscle cells in the wall of the arterioles enable these microvessels to constrict or dilate in response to nerve stimulation. The liver is richly innervated with nerve fibres. This innervation is through the anterior and posterior hepatic plexuses. The anterior plexus is the

smaller and lies in close association with the hepatic artery and is composed of sympathetic postganglionic fibres whose preganglionic counterparts arise from T7-T10 (Lewis, 1951). The posterior plexus lies at the posterior aspect of the portal vein. It includes a group of postganglionic sympathetic fibres from the right celiac ganglion. The preganglionic origin arises from T7-T10 as well. Both plexuses are interwoven (Lewis, 1951). The hepatic artery is innervated exclusively by the sympathetic fibres, whereas the bile ducts are innervated by both sympathetic and parasympathetic fibres (Rappaport, 1975). Electrical stimulation of the hepatic nerves leads to a marked decrease in hepatic arterial conductance (i.e. vasoconstriction) in a frequency-dependent manner (Greenway and Stark, 1971). This vasoconstrictor response is mediated by alpha adrenergic responses and can be completely blocked by phentolamine (Greenway and Stark, 1971). As with the arterial response, the portal vessels are under alpha adrenergic control which results in a rise in portal pressure when the hepatic nerves are stimulated (Lautt, 1979).

Other than its neural response, the hepatic artery responds with constriction or dilation to many pharmacological agents such as norepinephrine, epinephrine, angiotensin, vasopressin, glucagon, bile salts, isoproterenol, adenosine, histamine, etc. (Greenway and Stark, 1971). From a functional or physiological point of view, it is clear now that extrinsic

regulation of the hepatic microcirculation occurs mainly via the hepatic arterioles through nerve stimulation, hormones and bile salts (Rappaport, 1981).

It has been mentioned that the portal tract (i.e. the vascular stalk of the liver acinus) contains some lymphatic vessels. These lymphatics are part of the intrahepatic lymphatics. The intrahepatic lymph is drained through fine periportal venous and perihepatic venous channels. Both fine intrahepatic lymphatic routes communicate directly with the extremely large capsular lymphatics (i.e. lymphatics of Glisson's capsule) (Magari et al., 1979). The space of Disse has no direct communication with the lymphatics (Magari et al., 1979). This observation leads to the speculation that lymph passes from the space of Disse to the space of Mall (where lymphatic terminals are found) through gaps between the cells of the limiting plate. To the best of my knowledge, the data available on liver lymphatics do not prove or disprove the presence of functional relationships between periportal vein lymphatics and hepatic arterioles, nor between them and microvascular blood flow.

The circulation across the liver occurs at a low pressure gradient. The pressure drop from the terminal portal venules (5-9mmHg) to the hepatic veins (1-4mmHg) is less than 5mmHg. However, the pressure inside hepatic sinusoids is kept close to that of the portal vein through the existence of postsinusoidal resistance (Lautt, Greenway et al., 1986; Lautt

et al., (in press); Greenway et al., 1985). The postsinusoidal resistance site is within the small hepatic veins prior to their entry into the hepatic veins proper (Lautt, Greenway et al., 1986). It is essential to have a maintained basal level of sinusoidal pressure close to that of portal pressure to keep the integrity of a spongy organ like the liver and to prevent the collapse of some hepatic sinusoids. In addition to its role in maintaining the basal sinusoidal pressure, the postsinusoidal resistance inhibits or buffers to some extent the effect of the physiological fluctuations in central venous pressure (3-5.5mmHg) from being transmitted to the sinusoidal (lobar) or portal pressure (Lautt et al., (in press)). Mitzner (1974) previously suggested that hepatic postsinusoidal resistance acts as a Starling-resistor. The Starling-resistor waterfall model predicts that upstream blood pressures are absolutely protected against changes in downstream pressure until some critical pressure is reached and beyond that pressure, further increments in downstream pressure will be fully transmitted upstream. However, the situation is not so in the liver. The pressure transmission from central venous pressure (CVP) to sinusoidal (lobar) venous pressure begins with small rises in central venous pressure and becomes greater as the rise in central venous pressure increases. Changes in CVP in the face of higher postsinusoidal resistance will be weakly transmitted upstream but this is due to reduced proportion of transmission

over the entire pressure range and not due to having to overcome a higher waterfall pressure (Lautt, Legare et al., 1986).

At normal portal flow and pressure no significant presinusoidal resistance seems to exist (Lautt, Greenway et al., 1986). Some significant presinusoidal resistance can be generated and maintained if portal flow is greatly increased (Greenway et al., 1985) or when vasoactive agents such as norepinephrine and angiotensin are administered (Lautt et al., (in press)). However, the main rise in portal and sinusoidal pressure seen with hepatic nerve stimulation and vasoconstrictor hormone administration (such as norepinephrine and angiotensin) occurs at the postsinusoidal site within the hepatic veins (Lautt et al., (in press)).

1.4 HEPATIC CIRCULATORY HOMEOSTASIS AND LIVER FUNCTION

The stability of the hemodynamic and metabolic function of the liver is crucial to body homeostasis. Any change in the clearance function of the liver, for example, leads to change in plasma concentrations of many biological substances that can affect the well-being of the body. It seems from many observations that the stability of liver function depends on the maintenance of a stable intrahepatic pressure and a steady hepatic blood flow. Therefore, intrahepatic blood pressure and hepatic blood flow should be immune or as insensitive as possible to the passive effects of changes in

portal blood flow or cardiac output. Since the liver has no control over its portal flow, which is subject to changes from minute to minute, the only way for the liver to maintain its total blood flow is by changing its hepatic arterial flow. This relatioship is not a theoretical necessity, in fact it is a reality. It has been long observed that the hepatic arterial flow is consistently related to portal blood flow. As portal flow changes, hepatic arterial flow changes in the opposite direction to maintain steady total hepatic blood flow (Burton-Opitz, 1911). For further understanding of the important central role of the hepatic artery in maintaining stable liver function and thereafter in maintaining body homeostasis, related liver functions will be discussed individually.

1.4.1 The Capacitance Function of the Liver

The splanchnic bed is a major blood reservoir in the body. It is formed from three important components namely the liver, the intestine, and the spleen. In humans, it seems that the spleen is unimportant and the liver and intestine appear to be the important blood reservoirs (Price et al., 1966). A major portion of the splanchnic blood volume is contained in the hepatic venous circuit. The hepatic venous bed is under the control of the sympathetic nervous system (Greenway and Stark, 1971). Direct nerve stimulation results in a rapid and massive expulsion of blood from the hepatic venous circuit (up to 50% of the blood volume of the liver)

into the systemic circulation (Bennett et al., 1982). In acute hypovolemia, as during hemorrhage, the liver contracts and expells a volume of blood equal to 26% of the hemorrhaged volume regardless of the extent of hemorrhage (Lautt et al., 1980). Whereas, in hypervolemia the hepatic venous circuit accommodates 26% of the volume load. This indicates that the capacitance function of the liver as a blood reservoir is important to the cardiovascular homeostasis as a whole. However, it should be pointed out that intact nerve supply to the liver is not essential for the hepatic capacitance response to operate at normal hepatic venous pressure. Denervated or alpha adrenergically blocked liver can still expell blood in response to hemorrhage. Nephrectomy, hypophysectomy, and adrenalectomy also do not affect this response (Lautt et al., 1980). The decreases in hepatic blood volume are linearly related to reductions in hepatic blood flow regardless of the reason for that reduction. In other words, at normal hepatic venous pressure the hepatic capacitance function is regulated primarily by hepatic blood flow. Other factors that can affect the hepatic capacitance function (such as sympathetic nerve stimulation and vasoactive hormones) are redundant mechanisms. These redundant active mechanisms become the prime regulator of the capacitance response when the passive responses to decreased hepatic blood flow are reduced, such as during raised hepatic venous pressure (Lautt et al., 1980). It is not clear whether the

passive capacitance response is due to the change in hepatic blood flow per se or to the resultant change in the intrahepatic pressure. Recent studies suggest that intrahepatic pressure is the key factor in initiating the capacitance response (Bennett and Rothe, 1981; Greenway et al., 1985; Lautt et al., 1980). Liver capacity increases when hepatic venous pressure, and hence intrahepatic pressure, rises and portal flow is held constant (Greenway et al., 1985). However, no study overruled the role of hepatic flow per se on passive hepatic capacitance response. Such study should allow the measurement of changes in the hepatic reservoir capacity in response to changes in hepatic blood flow without changing the intrahepatic pressure. Regardless of the outcome of such study, the role of the hepatic artery in stabilizing the capacitance function of the liver is evident. Any local change in portal flow, and hence in hepatic blood flow, will be corrected for by changes in hepatic arterial flow in the opposite direction to keep the hepatic blood flow (and intrahepatic pressure) steady. Thence, liver volume will be held steady. Otherwise, any change in hepatic blood flow will be followed by an increase or decrease in the venous return to the heart. Such changes in venous return will be translated as change in cardiac output that will affect the whole cardiovascular homeostasis. Hence, through stabilizing the capacitance (reservoir) function of the liver, the hepatic artery stabilizes the

overall cardiovascular hemodynamics indirectly (Greenway, 1983).

1.4.2 The Clearance Function of the Liver

Hepatic clearance of a wide variety of compounds, including hormones, is blood flow dependent. Reduction of hepatic blood flow is known to decrease hepatic clearance of many compounds such as sulfobromophthalein, indocyanine green, collodial gold, galactose, lidocain, denatured albumin, colloidal chromium phosphate, oxyphenbutazone; and endogenous compounds such as aldosterone, cortisol, 11-deoxycorticosterone, corticosterone, and progesterone (Lautt, 1985). Since total hepatic blood flow determines hepatic clearance rates of many endogenous substances, including hormones, therefore, it is essential, for hormonal homeostasis, to have stable hepatic blood flow and it must not be allowed to be subject to the wide fluctuations of portal venous blood flow that occur continuously in response to postural and emotional changes and to variable metabolic states of the splanchnic organs. It has been mentioned above that the liver maintains its blood flow stable via changing the flow in the hepatic artery. Therefore, the hepatic artery controls indirectly the clearance function of the liver and its role is especially significant for the homeostasis of hormones which have very high turnover rates, i.e. where the rate of catabolism is an important determinant of the plasma levels of such hormones. Two unique characteristics of the hepatic circulation are

compatible with this putative role of the hepatic artery. First, the hepatic arterial flow is independent of liver metabolic activity (Lautt and Daniels, 1983; Lautt, 1980; Lautt, 1977b). When hepatic and intestinal metabolic rate is acutely stimulated (by use of dinitrophenol) oxygen uptake by the liver and gut increases. The gut responds to the increased oxygen demand by vasodilation resulting in increased portal flow and oxygen delivery to the gut. The hepatic artery shows no tendency for dilation despite the great reduction in portal and hepatic venous oxygen content and partial pressure (Lautt, 1980). However, the hepatic artery responds to the increase in portal flow by vasoconstriction resulting in holding the total hepatic blood flow constant. The liver maintains the high metabolic rate by increasing oxygen extraction in the face of reduced oxygen delivery (Edelstone et al., 1984; Lautt, 1980). Similar conclusions can be derived from the isovolemic hemodilution studies (Lautt, 1977b). In that study the oxygen delivery to the liver and gut is reduced. The arteries supplying the gut dilate and result in increased portal flow. Oxygen delivery to hepatocytes is decreased, however, the hepatic artery shows a small constriction and maintains total hepatic blood flow steady. Again, the hepatic metabolism is maintained as a result of increased oxygen extraction. Stimulation of liver parenchymal cell metabolic activity by low doses of taurocholate (bile salt) produces no vascular responses (Lautt

and Daniels, 1983). At higher doses the hepatic artery dilates in addition to the stimulation of parenchymal cell metabolism. The hepatic artery dilation is greater if bile salt is administered via the hepatic artery compared to that of portal vein administration. Therefore, the vascular and metabolic effects of bile salt are independent (Lautt and Daniels, 1983).

Compounds reaching the liver from the systemic circulation are equally accessible to hepatic parenchymal cells, regardless of their route of entry to the liver. Equal doses of taurocholate administered through the hepatic artery and portal vein are equally effective in stimulation of hepatic bile flow (Lautt and Daniels, 1983). Indocyanine green is equally well extracted as well by the liver regardless of its route of administration (Lautt et al., 1984). These two observations indicate that changes in hepatic arterial flow in response to changes in portal blood flow (to maintain steady hepatic blood flow, and thence, steady hepatic clearance) do not affect the pattern of intrahepatic irrigation of parenchymal cells. The liver normally receives much more oxygen than it requires and in cases of reduced hepatic arterial supply, the liver can readily extract more oxygen to compensate for altered delivery (Bredfeldt et al., 1985; Edelstone et al., 1984; Lautt, 1980). Therefore, it is rational to conclude that the hepatic artery, rather than being subservient to hepatic metabolic demands, is

the guardian of humoral clearance rates.

1.4.3 Stability of Intrahepatic Pressure

The hepatic artery seems to play an important role in stabilizing intrahepatic (sinusoidal) pressure. The venous circuit of the liver has no autoregulation capacity and the intrahepatic pressure therefore, depends on hepatic blood flow (Greenway et al., 1985; Richardson and Withrington, 1978). The relationship between intrahepatic pressure, hepatic blood flow and liver volume is linear (Greenway et al., 1985). The relationship between the hepatic arterial flow and portal flow, which tends to hold total hepatic flow steady, stabilizes the intrahepatic pressure. This has been demonstrated clearly in porta-systemic shunt procedures. In patients with portal hypertension and normal liver function tests, portacaval shunts cause an insignificant drop in portal pressure despite the significant decrease in hepatic portal inflow (Zimmon and Kessler, 1980). In these shunt procedures, the hepatic arterial flow increases to compensate for the decrease in portal flow and holds the total hepatic flow and intrahepatic pressure steady and close to their control levels. It was observed that failure of the hepatic artery to maintain the intrahepatic pressure after portacaval shunt procedure is a poor prognostic sign for patients with portal hypertension. In such patients portal pressure of 25cm H_2O (18mmHg) is a critical lower limit of pressure, below which hepatic function is not maintained and chronic encephalopathy

with early death supervenes (Zimmon and Kessler, 1980). The ability of the hepatic artery to hold portal pressure above 25cm H_2O after portacaval shunting, even if portal flow is retrograde, is a good prognostic sign and normal liver function is expected postoperatively (Zimmon and Kessler, 1980).

The existence of stable intrahepatic pressure is important for the stability of fluid exchange function of the liver. Elevated intrahepatic pressure produces a filtration of high protein fluid from the plasma to the surface of the liver and then into the peritoneal cavity (as with ascites that accompanies congestive heart failure and liver cirrhosis). This filtration is maintained for as long as the intrahepatic pressure is raised and it is directly proportional to the rise in intrahepatic pressure. It seems that there are no protective mechanisms to limit filtration that occurs in the liver as they do in the intestines and skeletal muscle (Greenway and Lautt, 1970). The sites of such filtration are still unclear. However, it is possible that fluid forces its way to the surface of the liver since hepatocytes are arranged in one-cell thick hepatic plates and fluid would pass from one space of Disse to another. Acute liver congestion and fluid filtration are accompanied usually by marked reduction in bile flow that does not recover for hours after the restoration of intrahepatic pressure (Greenway and Lautt, 1970). This may indicate disruption of the bile

canaliculi with passage of the bile into the plasma or lymph instead of into the bile ducts.

The rise in intrahepatic pressure during hepatic nerve stimulation is not associated with fluid filtration. On the contrary, hepatic nerve stimulation reduces the capillary filtration coefficient (Greenway, 1981). The possible mechanism is still unclear. It is anticipated that sympathetic nerve stimulation decreases sinusoidal permeability and closes the gaps in limiting plates between the space of Disse and the space of Mall which limits lymph outflow.

The stability of the intrahepatic and portal pressure is not esential for liver function alone. Changes in intrahepatic and portal pressure can be reflected reflexly or non-reflexly on the function of other organs too. Experimentally-induced presinusoidal portal hypertension is associated with an increase in intestinal blood flow (Groszmann et al., 1982). On the other hand, elevated intrahepatic pressure (as during liver congestion) is associated with a decrease in gut blood flow (Lautt, 1977a). These findings suggest possible existence of a liver-gut neural axis.

An increased portal pressure (less than 15cm H_2O) elevates urine flow rate within seconds, a response that can be blocked by renal nerve blockade (Ohm and Haberich, 1969). Portal pressure elevations beyond 15cm H_2O induce antidiuretic

effect (Liang, 1971). An increase in intrahepatic pressure, rather than an increase in portal pressure, produces a consistent reduction in urine flow, Na^+ excretion, and cortical renal blood flow (Levy, 1974). This effect, however, is believed to be a humoral and not a neural reflex since the restoration of renal function requires more than 30 minutes after the restoration of intrahepatic pressure (Levy, 1974). Regardless of the mechanism, the impact of intrahepatic and portal pressure on renal function is clear. This observation may explain the water and salt retention that accompanies hepatic congestion in congestive heart failure.

1.4.4 The Prevention of Hypoxemic State

The liver normally receives oxygen more than its requirement and can readily extract more oxygen to compensate for reduced delivery (Bredfeldt et al., 1985; Edelstone et al., 1984; Lautt, 1980). It has been mentioned above that the hepatic arterial flow is not regulated by hepatic parenchymal cell metabolism. Therefore, under normal conditions, parenchymal cells will not be adversely affected if hepatic arterial flow decreases to counterbalance an increase in portal flow. However, in situations where total hepatic flow is greatly reduced despite the compensatory increase in hepatic arterial flow (as during decreased cardiac output), oxygen's protective role of the hepatic artery is of paramount importance. It has been calculated that an increase in hepatic arterial flow to compensate just for 24% of the

decrease in portal flow is enough to hold total oxygen supply to the liver almost steady and offers protection from the hypoxemic state (Blumgart et al., 1977). The hepatic arterial protective role can be of importance as well, despite normal cardiac output, in other cases of reduced oxygen supply such as severe anaemia or being in a hypoxic environment.

1.5 THE HEPATIC ARTERIAL BUFFER RESPONSE

The relationship between the portal flow and hepatic arterial flow which tends to maintain steady total hepatic blood flow, has been described by many terms. The term 'reciprocal relationship' between the hepatic artery and portal vein has been used in some papers. In others the term 'autoregulation of the hepatic artery' was used. Nevertheless, such terms are not appropriate and do not reflect the true relationship between portal and hepatic arterial flows. Reciprocity implies that a decrease in portal flow is accompanied by an increase in hepatic arterial flow and vice versa. However, this relationship is operating in one direction. The hepatic artery responds to changes in portal flow, but portal venous flow does not respond to changes in hepatic arterial flow (Egbert and Raber, 1952). On the other hand, the term 'autoregulation' may be misleading since the term 'autoregulation of the hepatic arterial flow' is used as well to describe the pressure-flow relationship in the hepatic artery regardless of the flow in the portal vein.

The most appropriate terminology to describe the relationship between the hepatic artery and portal vein is 'the hepatic arterial buffer response'. This terminology was introduced by Lautt (1981) and defines the hepatic arterial buffer response as "the inverse control of the portal blood flow over hepatic arterial conductance independent of hepatic metabolic requirements". The term hepatic arterial buffer response will be used in this thesis. Figure 3 is an illustration of the hepatic arterial buffer response in the cat. The superior mesenteric arterial flow (SMAF) represents portal flow in this preparation. Point A and E represent the control state, point B represents the drop in SMA (portal) flow with the concommitant rise in the systemic (femoral) and hepatic arterial pressure and increase in hepatic arterial flow, point C represents the buffer response when the hepatic arterial pressure is returned back to the control level using an arterial clamp and there is still an increase in hepatic arterial flow, point D represents the release of the SMA occlusion (notice the SMA hyperemic overshoot and the corresponding drop in the hepatic arterial flow and systemic and hepatic arterial blood pressure). The significant roles of the hepatic arterial buffer response have already been discussed above under the subject of hepatic circulatory homeostasis and liver function.

From a historical perspective, the hepatic arterial buffer response was first noted by Burton-Opitz (1911),

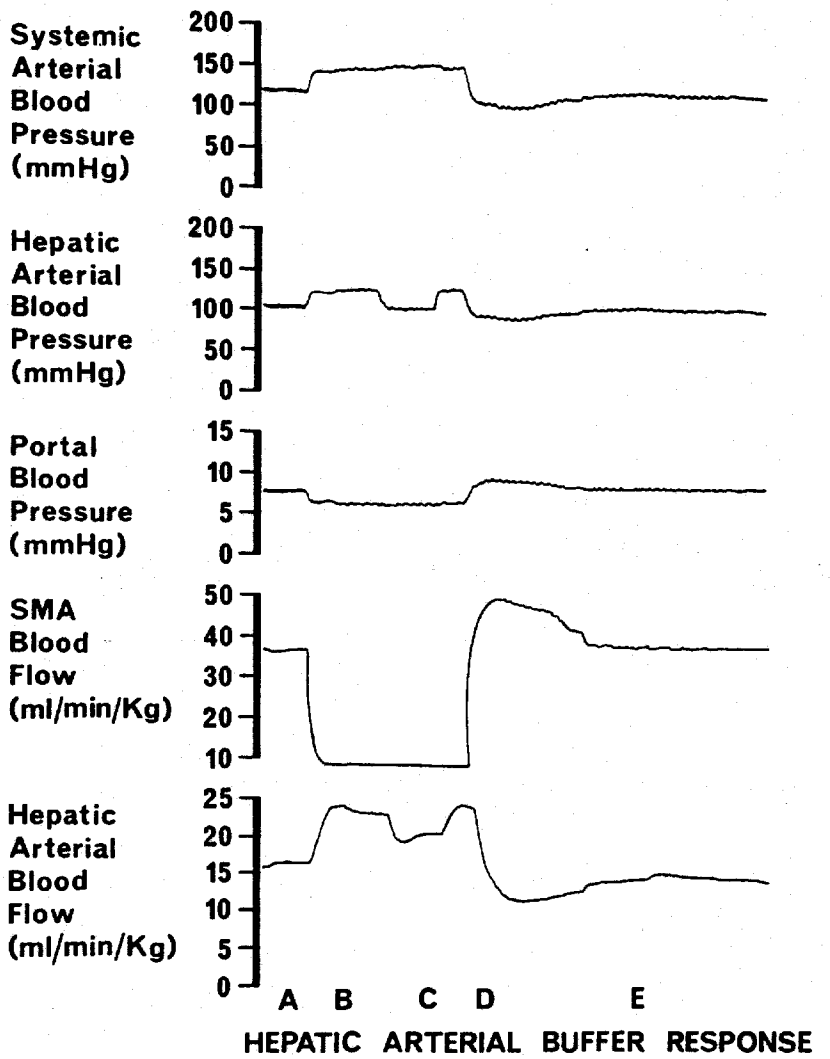

Fig. 3 Trace of the hepatic arterial buffer response taken from one anesthetized cat. Reduced superior mesenteric arterial flow (portal flow) results in a sudden rise in hepatic arterial flow in order to maintain stable total hepatic blood flow (for description of details refer to text). SMA = superior mesenteric artery.

followed by Barcroft and Shore (1912), when they tried to maintain a steady hepatic arterial perfusion pressure during the exclusion of the portal circulation. Since then the hepatic arterial buffer response received spotty attention with some authors in support and others being unable to detect the response. It seems that the methodology plays a significant role in confirming or denying such a response. Data derived from isolated perfused liver or from long-circuited hepatic artery preparations show absent or weak hepatic arterial buffer response (Burton-Opitz, 1911; Barcroft and Shore, 1912; Hanson, 1964; Hanson and Johnson, 1966; Sancetta, 1953; Shoemaker, 1964; Condon, Nyhus et al., 1962; Price et al., 1964; Takeuchi et al., 1966; Lutz et al., 1968). Observations made in preparations in which the hepatic artery was not long-circuited (i.e. intact) showed significant hepatic arterial buffer response in anesthetized (Ackroyd et al., 1966; Cohn and Kountz, 1963; Drapanas et al., 1960; Ternberg and Butcher, 1965) and conscious animals (Price et al., 1965). In human studies, the hepatic arterial buffer response has been observed as an abrupt compensatory increase in hepatic arterial flow during acute portal flow diversion at the creation of a portacaval anastomosis in portal hypertension patients (Zimmon and Kessler, 1980; Burchell et al., 1976; Schenk et al., 1962; Ferguson, 1963; Burchell et al., 1974). As mentioned previously, the failure of the hepatic artery to show the arterial buffer response after the

shunting procedure is a poor prognostic sign and an indication of a possible early postoperative death and encephalopathy (Burchell et al., 1976; Zimmon and Kessler, 1980).

The hepatic arterial buffer response can be observed in many experiments conducted on the liver, although such preparations were designed to study functions other than the hepatic arterial buffer response per se. The hepatic arterial buffer response is put in action when such experiments induce changes in portal flow. Some of such preparations will be discussed later. However, in other groups of experiments the role of the hepatic arterial buffer response can be concluded indirectly. Infusion of angiotenisn into the hepatic artery causes marked vasoconstriction and increases hepatic arterial resistance (Greenway and Stark, 1971). When angiotensin is administered by intravenous infusion, hepatic arterial flow decreases as well as portal flow due to intestinal and splenic vasoconstriction (Cohen et al., 1970). The vasoconstriction is less marked in the hepatic arterial bed than in the intestine and spleen, probably because the direct effect of angiotensin on the hepatic arterioles is counteracted by the hepatic arterial buffer response triggered by the fall in portal flow (Cohen et al., 1970). The same analogy can be used to describe the effect of vasopressin on the hepatic arterial bed. The direct action of vasopressin on the hepatic arterial bed is vasoconstriction but this is small and variable (Greenway and Stark, 1971; Peter et al., 1962;

Shoemaker, 1964). Intravenous infusions of vasopressin decrease portal flow, portal pressure and total hepatic flow due to vasoconstriction in the splenic and intestinal vascular beds (Cohen et al., 1970; Peter et al., 1962; Shoemaker, 1964), whereas hepatic arterial flow increases (Heimburger et al., 1960). It seems most likely that vasopressin has a weak vasoconstrictor effect on the hepatic arterial bed, but this vasoconstriction can be overcome by the dilator hepatic arterial buffer response initiated by the reduction in portal flow (Cohen et al., 1970).

Adenosine is a powerful dilator of the hepatic artery and the superior mesenteric artery. However, intravenous infusions of adenosine increase portal flow and pressure (by intestinal vasodilation) and cause negligible changes (Greenway and Stark, 1971) or marked decrease (Lagerkranser et al., 1984) in the hepatic arterial flow. Contrary to the effect of vasopressin and angiotensin, it seems that adenosine increases portal flow and thence initiates a vasoconstrictor hepatic arterial buffer response. This vasoconstriction may neutralize or overcome the direct dilator effect of the circulating adenosine on the hepatic artery.

The impact of the hepatic arterial buffer response can be predicted as well from the hemorrhage-induced hemodynamic changes in the liver. After hemorrhage, portal flow decreases due to arteriolar vasoconstriction in the spleen and intestine. The splenic vasoconstriction is due to activity of

the sympathetic nervous system (i.e. the increase in sympathetic tone), angiotensin and vasopressin (antidiuretic hormone, ADH) (Greenway and Stark, 1969). In the intestinal vascular bed, the vasoconstriction is due principally to the actions of angiotensin and vasopressin, while the increase in sympathetic tone plays at most a minor role (McNeil et al., 1970). The decrease in portal flow is proportionally greater than the decrease in total hepatic flow. Hepatic arterial flow is either unchanged or increased in spite of a fall in arterial pressure; hepatic arterial resistance consistently decreases (Greenway and Stark, 1971). The mechanism of the vasodilation is not clear. However, it is highly suggestive that the reduction in portal flow stimulates the hepatic arterial buffer response to induce dilation of the hepatic artery. The existence of other factors which contribute to the dilation cannot be excluded at this stage.

What is the mechanism of the hepatic arterial buffer response? Many hypotheses have been suggested by many authors. These hypotheses are illustrated in Figure 4. The physical analog has been suggested (Ternberg and Butcher, 1965) and considers the hepatic arterial buffer response as the mechanical effect of the interposition of a slower flowing portal stream into the path of a faster flowing arterial stream. Decrease in portal flow is equivalent to removal of an impedance, and the rate of flow in the hepatic artery should increase. Conversely, if portal flow increases, its

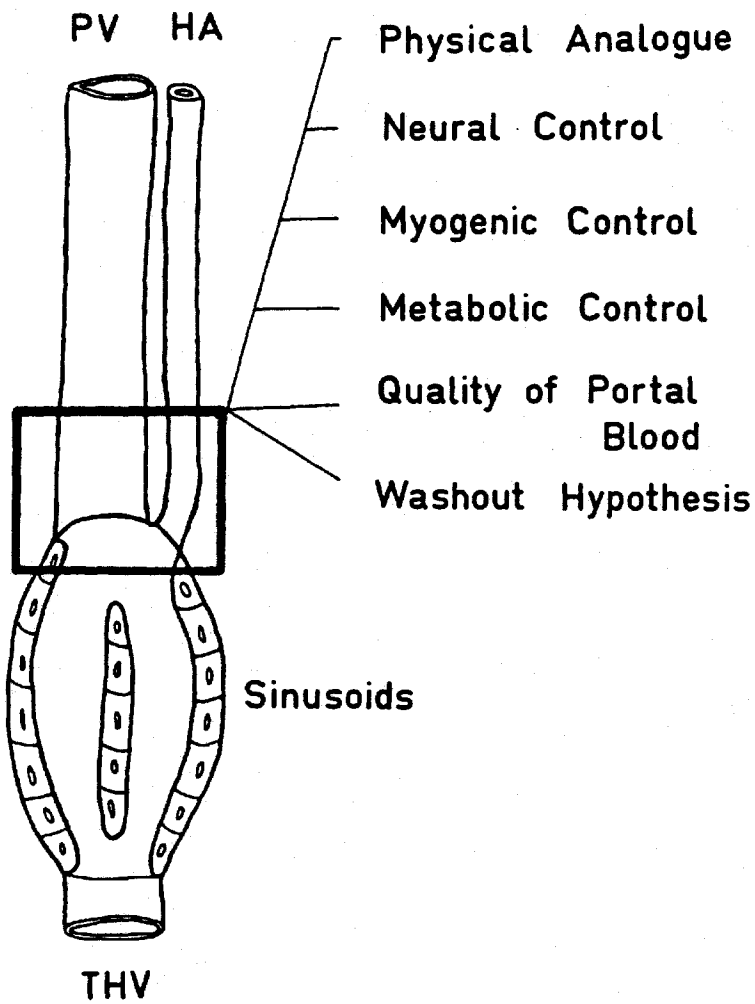

Fig. 4 The possible mechanisms for linking blood flow control in the hepatic artery (HA) to flow in the portal vein (PV) such that total hepatic flow (THV) remains constant.

impedance to the hepatic arterial flow increases as well, therefore, a decrease in hepatic arterial flow should result. Such a mechanical hypothesis implies that the hepatic arterial flow is an impetus to the portal flow and an increase or decrease in the hepatic arterial flow produces an increase or decrease in portal flow respectively. This mechanical hypothesis cannot be accepted for two reasons. First, many researchers failed to detect the hepatic arterial buffer response in isolated perfused liver or long-circuited hepatic artery preparations as mentioned above. Second, occlusion of the hepatic artery does not cause a significant change in portal flow in normal humans during surgery nor in patients with portal hypertension (Egbert and Raber, 1952). My personal observation in our laboratory supports this finding.

The neural control hypothesis implies that such control of the buffer response could occur via an extrahepatic and/or an intrahepatic nerve network. Complete extrinsic denervation does not eliminate the buffer response (Lautt, 1981; Mathie et al., 1980; Sancetta, 1953). There is no anatomical evidence to support the existence of an intrinsic hepatic nerve network. Moreover, the hepatic arterial buffer response is absent in the isolated perfused liver while the vasculature is able to respond normally to the vasoconstrictor effect of the hepatic sympathetic nerves when electrically stimulated. It seems highly unlikely that such a preparation prevents function of an intrinsic nerve network while still allowing

the extrinsic nerves to function normally.

The myogenic control hypothesis was believed to be the mechanism behind the hepatic arterial buffer response for some time (Hanson and Johnson, 1966). Such a hypothesis suggests that a rise in transmural pressure across the arteriolar wall causes an increase in the rhythmic myogenic activity of the smooth muscle in the wall. The overall effect of such an increase in activity is vasoconstriction (Mellander and Johansson, 1968). In other words, such a hypothesis is based on changes in the sinusoidal pressure to explain vasoactive changes in terminal hepatic arterioles. A rise in sinusoidal pressure (as a result of an increase in portal blood flow) causes hepatic arterial vasoconstriction and a fall in sinusoidal pressure causes vasodilation. However, recent studies suggest that the hepatic arterial flow changes in response to changes in portal flow despite the stability of portal (and thence sinusoidal) pressure. Hemodilution causes a rise in portal flow but portal pressure remains unchanged (Lautt, 1977b). There is correlation between changes in arterial flow and changes in portal flow but not with changes in portal pressure or arterial pressure. The stability in portal venous pressure is maintained as a result of increased vascular conductance within the liver. Similarly, the stimulation of gut metabolism by dinitrophenol causes elevation in portal blood flow while portal blood pressure remains steady (Lautt, 1980). The hepatic artery constricts

to offset elevated portal venous blood flow, thereby maintaining total hepatic blood flow constant. Portacaval shunting procedure in some portal hypertensive patients results in decreased portal venous flow, increased hepatic arterial flow and stable prehepatic portal venous pressure (Zimmon and Kessler, 1980). The existence of the hepatic arterial buffer response despite the stability of portal (and sinusoidal) pressure contradicts the explanation of the myogenic hypothesis of the hepatic arterial buffer response. However, these data do not exclude the possible existance of a myogenic control for the hepatic artery.

The metabolic control hypothesis is based on the formation of vasoactive byproduct by hepatocytes in response to reduced portal flow. On the other hand, an increase in portal flow will be translated as less hypoxia and less formation of vasoactive metabolic byproduct. However, it is evident from the previous discussion that the hepatic artery does not function in a similar manner to other arteries; i.e., the hepatic arterial blood flow is not controlled by the local metabolic needs of hepatocytes and does not dilate in response to reduced oxygen delivery. The microanatomy of the liver supports this physiological finding. All blood flow inputs enter the acinus at the centre; flow in adjacent sinusoids is concurrent, and all of the exit points are adjacent with no opportunity for shunting of oxygen (from zone 1 to zone 3 where blood drains into terminal hepatic venules). Similarly,

back diffusion of metabolites from the periphery of the acinus to its central part, where the resistance vessels controlling blood flow are located, is also precluded, thus preventing a dilator substance in the venous blood from affecting the hepatic arterioles upstream.

The quality of portal blood hypothesis suggests the possible existence of a vasoconstrictor substance released into portal blood by splanchnic organs. Elevation of portal flow might then deliver more constrictor to the hepatic vasculature and result in arterial constriction. Reduction of portal flow, on the other hand, means delivery of less constrictor to the hepatic arterioles and dilation should result. Such a hypothesis can be tested by replacing portal blood by vena caval blood. A pilot study has been performed in our laboratory where crossover experiments allow the portal blood to be shunted into the vena cava and the vena caval blood to be shunted into the portal vein (Lautt, 1981). The shunt could be shifted quickly so that the liver could be perfused with either portal or vena caval blood. The buffer response was equally sensitive regardless of whether the liver was perfused with portal or vena caval blood. Thus the factor controlling the hepatic arterial buffer response appears to be blood flow per se and not its quality.

One hypothesis remains to be fully evaluated, the dilator washout hypothesis. This hypothesis proposes that a dilator substance is produced in the vicinity of the smooth muscle

cells of the hepatic arterial resistance vessels in the space of Mall. The dilator is free to interact with receptors on the muscle or to diffuse out of the cell and be washed away by the bloodstream (Figure 5). Reduction of portal blood flow would result in less washout and the accumulated dilator substance would result in arterial dilation. Conversely, an increase in portal blood flow would result in more washout and the low concentration of the dilator substance would result in arterial constriction. Such a hypothesis implies that portal blood must have access to the hepatic arterial resistance vessels so that portal flow can wash away such dilator from the area of the resistance vessels. However, this condition is readily fulfilled. Infusion of vasoactive substances into the portal blood results in effect on the hepatic artery and vice versa (Richardson and Withrington, 1981). Although the anatomical relationship may be unclear, it is evident that portal blood does have access to the hepatic arterial resistance vessels. To test the dilator washout hypothesis three criteria must be met: 1) the substance that can be washed away by portal blood must dilate the hepatic artery; 2) potentiators of the exogenous doses of such a dilator should also potentiate the buffer response; 3) blockers of the exogenous doses of such a dilator should also inhibit the buffer response.

What is the dilator substance? In a series of preliminary experiments conducted in our laboratory in attempt

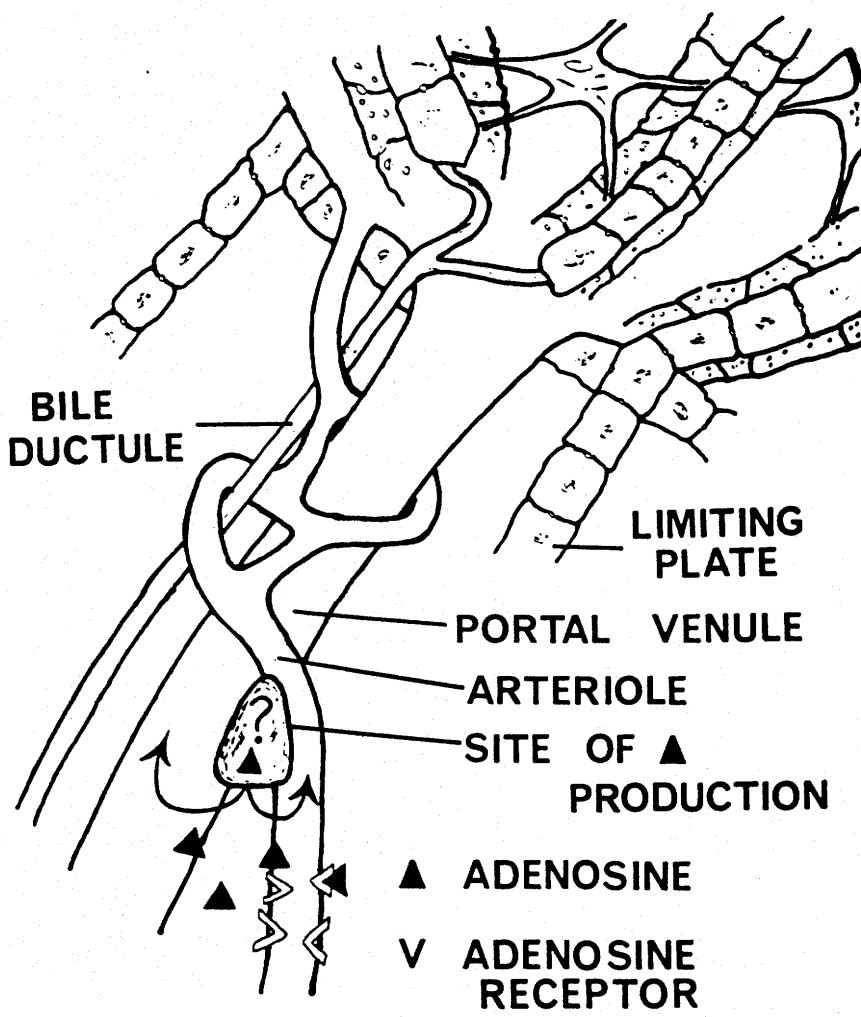

Fig. 5 Schematic description of the washout theory: Locally produced adenosine has access to the hepatic portal and arterial resistance sites in the presinusoidal vicinity. Decreased hepatic blood flow leads to less wash out and accumulation of adenosine that results in hepatic arterial vasodilation. The effect of altered portal blood flow on local adenosine concentration accounts for the mechanism of hepatic arterial buffer response. The effect of altered hepatic arterial blood flow on local adenosine concentration accounts for the mechanism of hepatic arterial autoregulation. (Lautt et al., 1985).

to interfere with the hepatic arterial buffer response by using blockers or potentiators, it appears that atropine, propranolol, ouabain, aminophylline, theophylline, indomethacin, metiamide and mepyramine do not interfere significantly with the normal buffer response (Lautt, 1983). One compound, dipyridamole (an adenosine uptake inhibitor), has been shown to enhance the buffer response. Although such observation needs further support, it nevertheless suggests that the dilator may be adenosine (Lautt, 1983). This suggestion is attractive. It is well known that after hemorrhage, peripheral resistance increases due to generalized arteriolar vasoconstriction. However, three arteries are spared from the constrictor response and exhibit dilation. These arteries are namely the cerebral, the coronary and the hepatic artery. After hemorrhage the fractional distribution of cardiac output is progressively increased to brain, heart and hepatic arterial vascular bed at the expense of other organs like skin, spleen, pancreas, bones, etc. The hepatic artery vascular bed is the only one that shows significant vasodilation at different levels of hemorrhage (Forsyth et al., 1970; Slater et al., 1973; Bellamy et al., 1984; Kaihara et al., 1969; Gerkens et al., 1982). Adenosine has been proposed as a metabolic regulator of coronary (Berne, 1963; Thompson et al., 1980) and cerebral blood flow (Berne, 1985) during hypotension and hypoxia. However such regulation has not been confirmed physiologically. Could it be possible that

adenosine is the universal blood flow regulator of these three particular arteries? Such a hypothesis needs to be tested and fully evaluated.

By exclusion it seems that the dilator washout hypothesis is the only hypothesis that can explain the mechanism of the hepatic arterial buffer response without major objections.

1.6 HEPATIC ARTERIAL PRESSURE-FLOW AUTOREGULATION

It has been mentioned above that the hepatic arterial conductance changes in response to variation in portal blood flow (i.e. the hepatic arterial buffer response) in a way to keep total hepatic blood flow steady. Regardless of portal flow, can the hepatic artery change its own conductance in response to changes in its perfusion pressure? Over the years, many studies have been carried out on the effects of changes in hepatic arterial pressure on hepatic arterial flow. If no active changes in the vascular bed occur, a graph of flow against pressure is linear or convex to the pressure axis. A graph that is concave to the pressure axis indicates an active response of the resistance vessels, and such a mechanism tends to maintain blood flow and capillary pressure constant when changes occur in arterial pressure. This mechanism is referred to as hepatic arterial autoregulation. The term autoregulation has previously been used loosely to describe two different situations, the change in hepatic arterial resistance induced by a change in the portal flow and

the change in hepatic arterial resistance in response to a change in its perfusion pressure. In the first case, and as it has been described in section 1.5, the term hepatic arterial buffer response (HABR) was coined. The term "autoregulation" will be used in this thesis to describe the ability of the hepatic artery to maintain a relatively constant flow in the face of changes in its perfusion pressure, independent of the portal flow.

The existence of pressure-flow autoregulation in the hepatic arterial bed is a controversial issue. Some studies have demonstrated autoregulation (Andrews, 1958; Condon, Chapman et al., 1962; Greenway et al., 1967; Takeuchi et al., 1966; Torrance, 1961), although its capacity may be small and not seen in all experiments (Hanson, 1973; Hanson and Johnson, 1966). Other studies have indicated the absence of autoregulation in the hepatic arterial bed, with the hepatic arterial resistance either remaining constant or decreasing with increasing arterial pressure (Richardson and Withrington, 1978; Shoemaker, 1964). This controversy cannot be explained on the basis of the experimental design or the state of innervation of the liver.

The mechanism behind hepatic arterial autoregulation was believed to be a myogenic one (Hanson, 1973; Hanson and Johnson, 1966). The ability of papaverine, a potent direct smooth muscle relaxant, to abolish hepatic arterial autoregulation was considered as support for the myogenic

hypothesis (Hanson, 1973). However, a potent vascular dilator like papaverine can mask the effect of locally produced vasodilators as well. Therefore, papaverine-induced inhibition of autoregulation cannot differentiate mechanisms. The previous information led us to the conclusion that the mechanism behind hepatic arterial autoregulation is still unclear.

1.7 THE OBJECTIVES OF THIS RESEARCH WORK

It is evident from the above introduction that the hepatic artery plays a central and fundamental role in liver function and thence body homeostasis as a whole. However, major unsolved questions remain. The hepatic arterial buffer response has been noted by many researchers, nevertheless, no method to quantitate this buffering capacity has been suggested. Therefore, the first aim of this research work was to propose a method to quantitate the hepatic arterial buffer response and to test whether the buffering capacity is constant, regardless of the change in portal blood flow, or if it is a variable one. The second objective of this research work was to test the dilator washout hypothesis and to determine if adenosine is the dilator responsible for the mechanism of the hepatic arterial buffer response. The third goal of this work was to confirm or deny the existence of hepatic arterial pressure- flow autoregulation and to find the best method to evaluate autoregulation and whether the present

controversy regarding the existence of autoregulation can be accounted for by the methods of evaluation previously used. If autoregulation is present, the fourth objective was to determine the mechanism of autoregulation and whether it can be explained by the adenosine washout theory, i.e. has the hepatic arterial flow, like the portal flow, access to wash out adenosine?

2. METHODS

2.1 SURGICAL PREPARATION

Two series of experiments have been conducted, one to test the quantitation and the mechanism of the hepatic arterial buffer response, the second to test the hepatic arterial pressure-flow relationship and its mechanism. The surgical preparation was basically the same in both series with some minor modifications to suit the objectives of each series.

Cats of either sex were anesthetized with sodium pentobarbital, 32.5mg/Kg body weight (Somnotol - M.T.C. Pharmaceuticals) given intraperitoneally. The level of anesthesia was maintained by supplementary doses of 6mg intravenously when corneal or swallowing reflex seemed to recover. The body temperature was maintained at 37.5^{o}C by use of a rectal probe and a thermal controlled unit (Yellow Springs Instruments, model 72) operating heating tapes in the surgical table. The cats were fasted overnight with free access to drinking water.

A polyethylene cannula (PE90) was placed in the brachial vein for administration of anesthetic and fluids as required. The plasma replacement solution was composed of Ringer's solution and 10% Gentran 40 (Dextran 40) along with 5% Dextrose in the ratio of 1:1. This solution was maintained at 37^{o}C by using a water bath. Another cannula was placed in the right femoral artery to monitor the systemic arterial blood pressure. After laparotomy and splenectomy, the celiac artery

and its branches (i.e. the common hepatic artery, splenic artery, gastric artery, etc.) and the superior mesenteric artery were identified. Two electromagnetic flowprobes were placed to measure hepatic arterial blood flow (HAF) and superior mesenteric arterial blood flow (SMAF). Figure 6 shows the vascular preparation used. The hepatic arterial probe (Carolina Medical Electronics EP406) was placed on the celiac artery and all flow in the celiac artery was directed into the hepatic artery by ligating all branches of the celiac artery as well as the gastroduodenal artery. The gastroduodenal artery and the splenic artery were cannulated to allow for close intrahepatic arterial infusion of drugs through the former and monitoring of hepatic arterial perfusion pressure via the latter.

A micrometer-controlled screw clamp was placed around the celiac artery distal to the flowprobe to control flow and pressure within the hepatic artery. The second flowprobe (Carolina Medical Electronics EP408) was placed on the superior mesenteric artery (SMA). In this preparation, the SMA flow is synonymous with portal blood flow as all other inlet arteries that supply the portal vein (i.e. inferior mesenteric artery, gastric artery, gastroduodenal artery, and splenic artery) were ligated. In the autoregulation series, an inflatable vascular occluder on the SMA was used, distal to the flowprobe to determine the zero baseline and to initiate the hepatic arterial buffer response (HABR). In the

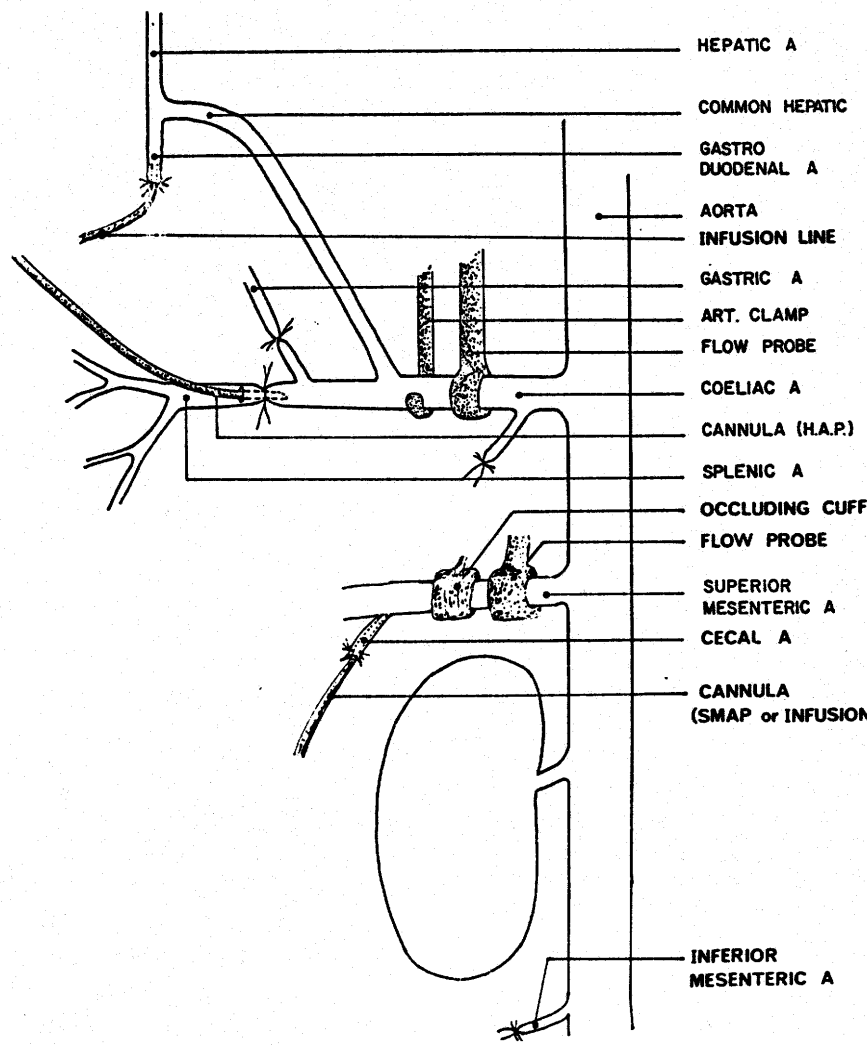

Fig. 6 Schematic description of the surgical preparation. Portal flow to liver is made equal to superior mesenteric arterial flow by removal of spleen, occlusion of gastroduodenal artery, inferior mesenteric artery and gastric artery. See text for calibration procedure. A, artery; SMAP, superior mesenteric arterial pressure. (Lautt et al., 1985).

quantitation series, on the other hand, the inflatable vascular occluder on the SMA was replaced by another micrometer-controlled screw clamp to allow stepwise occlusion of the superior mesenteric arterial blood flow (SMAF).

This methodology allows measurements of blood flows in the only arteries left perfusing the liver. All blood entering the portal vessel must derive from the superior mesenteric artery. The anastomotic connections of the SMA provide adequate blood flow to the regions normally supplied by the gastric and inferior mesenteric arteries as judged by lack of gross evidence of ischemic regions and the presence of microspheres in all tissues when injected via the ascending aorta (19 observations) (for methodology of injection and results see section 2.3 and Appendix B). On three occasions, postmortum evaluation of the surgical preparation was conducted. The thoracic aorta and the superior mesenteric artery were ligated and cannulated. Pink latex was injected through the superior mesenteric arterial line to visualize the area of its blood supply. Blue latex was injected in the descending thoracic aortic line. The blue latex filled the abdominal aorta and the celiac artery and all their tributaries. The cat carcass was stored in the cold room and macroscopic examination was conducted 24 hours later. Latex-injected dissections revealed no blue latex (non-SMA tributaries) in the splanchnic organs except at the cardia of the stomach and the lower end of the rectum. This finding has

confirmed a lack of non-SMA inlets to the splanchnic organs in our preparation.

A catheter was advanced from the small vein that drains the cecum into the portal vein, the tip of which was about 2cm back from the hilum of the liver, to allow for continuous portal venous pressure measurement. Another catheter was advanced from one of the splenic veins into the portal vein. The tip of this catheter was 2.5-3cm back from the hilum of the liver to allow for close intrahepatic portal infusion of drugs with adequate mixing in the portal blood (Lautt et al., 1984).

In a group of cats from the autoregulation series, a snare was placed around the abdominal aorta below the kidneys. The occlusion of the abdominal aorta at this position will increase the peripheral resistance and elevate systemic blood pressure. Since occlusion of the aorta below the kidneys interferes with systemic pressure recording from the femoral arterial line, in this group of cats the superior mesenteric arterial pressure was monitored via a cannula placed in the artery supplying the cecum. Gould and Statham pressure transducers were used for measuring blood pressure; blood pressure and flows were recorded on a Sensor Medics RM611 dynograph.

2.2 CALIBRATION

Prior to the commencement of experiments pressure transducers were calibrated. A glass manometer, U in shape, partially filled with mercury or water was used to calibrate for arterial and venous pressure respectively. One of the two open limbs of the manometer was connected by a rubber tube to the pressure transducer via a three-way stopcock. With the occlusion of the connection between the transducer and the manometer, air was pumped (by a syringe) through the stopcock to the manometer to create a column difference of mercury equal to 100mm (10cm). Then the connection between the manometer and the transducer was reopened at the stopcock site. The signal recorded by the dynograph recording pen was then adjusted, with the use of special amplifiers, to make a 20mm deflection from the preset zero baseline. Hence, every 1mm deflection on the dynograph record is equivalent to 5mmHg pressure. This transducer was allocated to measure the arterial blood pressure. Technically, the same procedure was used to calibrate the transducer used to measure venous pressure with some changes. The mercury was replaced by water and the column difference was set to be 13.6cm (equal to 10mmHg). Therefore, 1mm deflection from the venous pressure transducer is equal to 0.5mmHg. Both arterial and venous pressure transducers were set on the surgical table at the level of the inferior vena cava of the animal. Pressure transducer calibration was rechecked every 6-8 weeks.

The calibration of each electromagnetic flowprobe was conducted in situ at the conclusion of each experiment. The calibration procedure of the hepatic artery was facilitated by the application of a thread placed around the common hepatic artery during the initial surgery. This thread was pulled to give a zero flow at the conclusion of the surgical setup. This zero flow must match the zero flow obtained using the clamp on the celiac artery. This test is done at the end of each experiment as well. Then the polarity on the flowmeter (Carolina Medical Electronics, electromagnetic blood flowmeter, Model 501) was reversed and the animal's own blood was pumped at a constant rate, using a Harvard infusion pump, backward through the celiac artery using the catheter cannulated through the splenic branch of the celiac artery (the catheter that was used to monitor hepatic arterial pressure). The advantages of this system are that calibration can be done in situ without need to open the abdomen, and the constant infusion rate (38ml per minute, from 50ml syringe) allows for an absolutely stable and precise measurement to be made from the flow trace with a precisely known flow rate.

The approach to the SMA calibration was traditional. The abdomen was reopened at the conclusion of the hepatic artery calibration, and the SMA was cannulated distal to the flowprobe. The blood was then infused backward (i.e. toward the aorta) with reversed flowmeter polarity. The reopening of the abdomen and the perturbation of the flowprobe makes the

calibration less ideal compared to that of the hepatic artery. The blood used for calibration was obtained by withdrawing 50-80ml of blood from the cat at the end of the experiment. The blood was kept in a beaker with heparin (1000-3000 IU) to prevent coagulation.

2.3 THE RADIOACTIVE MICROSPHERE TECHNIQUE

The microsphere technique was used in few animals (n=5) to validate the use of the superior mesenteric arterial flow (SMAF) as an index of portal blood flow. This technique allows the measurement of regional blood flow changes in conscious and unconscious preparations without inducing significant hemodynamic alterations. The principle behind this technique is that proper mixing of plastic microspheres with the blood stream leads to even distribution of these microspheres within the blood stream, and ends in their permanent entrapment in the arterioles or capillaries (i.e. microembolization) on the first circulation after injection. Hence, microsphere distribution would approximate red blood cell distribution and the percentage of microspheres entraped in a tissue would be equal to the percentage of cardiac output supply of blood flow to that tissue. The proportion of distribution of microspheres can be obtained by substituting the number of microspheres by its corresponding radioactivity since the number of entraped microspheres is directly proportional to the amount of radioactivity they emit (Lebrec

and Blanchet, 1985; Heymann et al., 1977). It is inconvenient to give the full description of the radioactive microsphere technique in this thesis since the pilot study, in which it was used, was designed to validate a technical point in our surgical preparation. The full description of the technique was dealt with elsewhere (Heymann et al., 1977). Briefly, the surgical preparation was identical to that used in the quantitation and autoregulation series with minor modifications. The right carotid artery was cannulated with a PE190 cannula. The cannula was advanced retrograde 8.5cm toward the arch of the aorta. The slightly precurved tip of this cannula was placed in the arch of the aorta facing the aortic valve of the left ventricle. This cannula was used to administer the radioactive-labelled microspheres. Postmortum examination of each cat was performed to check the position of the carotid cannula. Then the kidneys, lungs, liver, pancreas, stomach, small intestine, large intestine, rectum and omentum were taken and their weight (in grams) and radioactivity were measured.

Two kinds of radionuclide-labelled microspheres were used in this study. They were Cobalt 57 (^{57}Co, New England Nuclear, Cat. No. NEM 012A, Lot. No. Co1569, size 16.4 $\pm$ 0.2u) with a specific activity of 11.52 mCi/g and a half life of 271 days, and Tin 113 (^{113}Sn, New England Nuclear, Cat. No. NEM 062A, Lot. No. SN1568, size 16.5 $\pm$ 0.1u) with a specific activity of 12.11 mCi/g and a half life of 115 days. The

approximate number of microspheres administered in each experiment was 500,000 microspheres of each kind. All radioactivities were counted by using LKB WALLAC 1282 Compugamma (Universal Gammacounter). Each batch of microspheres was placed inside a glass injector (Figure 7). The microsphere suspension was mixed (by vortex) for 1 min before the injection of microspheres into the body of the cat with 5ml warm, normal saline (37^{o}C) through the carotid line. The injection of microspheres was manual over a period of 15-25 seconds along with continuous shaking of the injector. A reference blood sample was withdrawn from the femoral arterial cannula at a rate of 1.94ml/min using a Harvard infusion-withdrawal pump. The withdrawal started 15-20 seconds before the administration of microspheres and lasted 1.5 minutes after the end of microsphere infusion. Tissue samples and reference blood samples were counted all on the same day. The spill-over correction of ^{57}Co and ^{113}Sn activity as well as decay correction were made automatically by the computerized unit attached to the gammacounter.

The protocol of this microsphere study was to administer the first batch of microspheres (control) to be followed, 5-10 minutes later, by occlusion of the SMA and administration of the second batch of microspheres. The detection of any radioactivity, from the second batch of microspheres, in the splanchnic organs would suggest the existence of an arterial contribution to portal flow other than the SMA flow.

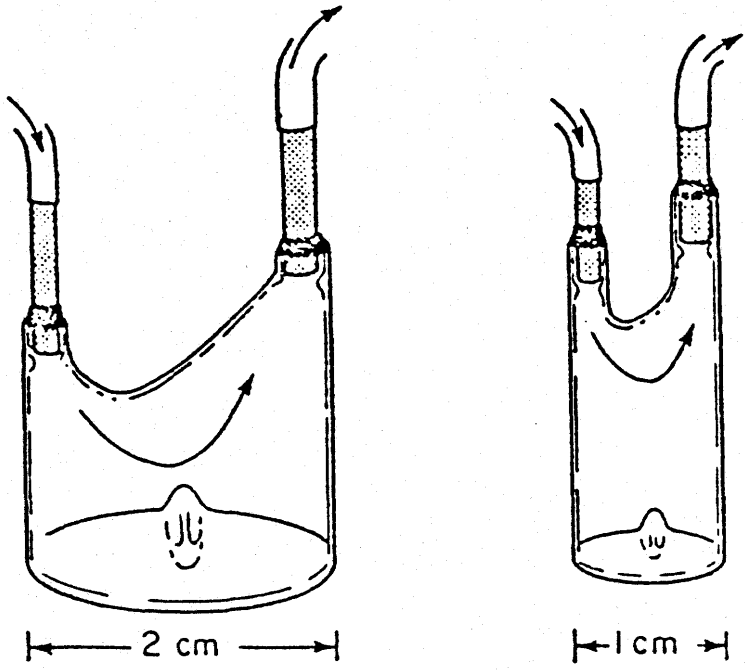

Fig. 7 Diagramatic illustrations of the glass vials used to inject the microspheres. The size used depends on animal size and hence the number of microspheres to be injected, the flushing volume and the size of the gamma-counter vial. (Heymann et al., 1977).

Calculation of regional blood flow to any organ was made according to this formula:

$$\text{Organ flow (ml/min)} = \frac{\text{organ radioactivity x reference sample withdrawal rate}}{\text{reference sample radioactivity}}$$

The radioactivity of an organ should be of the same range of nuclide activity as the reference blood sample. The same formula was used to calculate cardiac output (CO) with the substitution of the organ activity by the total activity administered to the animal. Decay correction calculation was made according to the formula:

$$A_o = A \times 2^{\frac{h}{H}}$$

Where A_o is the original radioactivity and A is the corrected radioactivity; H is the isotope half life and h is the time elapsed between microsphere administration and tissue samples counting. Portal blood flow was calculated from the sum of flows to the splanchnic organs. These organs are the stomach, small and large intestine, rectum, pancreas and the omentum. The contribution of these organs to portal flow was confirmed by retrograde infusion of blue-latex in the portal vein on three occasions. The pattern of distribution of the latex indicated that portal vein tributaries arise between the lower end of the esophagus (about 2cm above the cardia of the stomach) and the last 2-3cm of the rectum including the pancreas and the omentum.

The lungs were examined for the presence or absence of radioactivity to test for the existence of microsphere shunting from the arterial to the venous circulation. Equal renal flow for each kidney was an index of proper mixing of microspheres with the blood stream.

2.4 SOLUTIONS AND CHEMICALS

Adenosine was dissolved in warm Ringer's solution and infused at a rate of 0.206ml/min ($0.4mg.min^{-1}.Kg^{-1}$). The infusion was through the portal vein. Three adenosine receptor blockers have been used. 1-Methyl-3-isobutyl-xanthine (MIX, Sigma Chemical Company, No.1-5879) and 8-phenyltheophylline (8-PT, Research Biochemicals Inc., Cat. No. A-12) each was dissolved in warmed NaCl (0.1M) solution at a pH of 12.27 adjusted with 1N NaOH. MIX was used in the quantitation series and was infused at a rate of 0.34ml/min ($0.1mg.min^{-1}.Kg^{-1}$). The third adenosine receptor blocker 8(p-sulfophenyl) theophylline (8-SPT, Research Biochemicals Inc., Cat. No. A-13) is water soluble and was dissolved in warm Ringer's solution. The two adenosine receptor blockers, 8-PT and 8-SPT, were used in the autoregulation series and were given in bolus doses and not as continuous infusion as with MIX. The route of administration of the three adenosine receptor blockers was into the hepatic artery to get the highest chance of contact between the blockers and adenosine receptors in the hepatic arterial vascular bed.

In some experiments of the autoregulation series norepinephrine (Sigma Chemical Co., L-Arterenol HCl, No. A-7381) and isoproterenol (Sigma Chemical Co., L-isoproterenol HCl, No. I-6504) were infused intraportally at a rate of 0.1ug.min^{-1}.Kg^{-1} body weight and 0.2ug.min^{-1}.Kg^{-1} body weight respectively. Each was dissolved in Ringer solution and infused at a rate of 0.136ml/min. Norepinephrine was prepared from stock solution containing 1mg/ml norepinephrine (NE). Norepinephrine comes from the supplier as salts of NE-bitartrate (mol. wt. = 337.3) or NE-HCl (mol. wt. = 205.7). The actual molecular weight of norepinephrine itself is 169.2. Therefore, to make 1mg NE/ml stock solution, 1.22mg of NE-HCl should be dissolved in 1ml of normal saline or distilled water. For catecholamines there is a danger of oxidation during storage, so 10mg of ascorbic acid (a stabilizer) was added to each 50ml of the stock solution before the volume is reached. Then the stock solution of norepinephrine is divided into small vials and stored frozen in a deep freeze (-5°C). To calculate the concentration and volume of NE solution required for each experiment these general formulas were used:

A) dose required (mg.min^{-1}.Kg^{-1})/pump infusion rate (ml.min^{-1}) = Xmg.ml^{-1}.Kg^{-1} = concentration required expressed per Kg body weight.

B) stock conc./conc. required = dilution factor.

C) stock volume to be diluted = mls of NE-solution sufficient for the exp./dilution factor.

2.5 QUANTITATION OF THE HEPATIC ARTERIAL BUFFER RESPONSE

In a group of cats (n=7) the superior mesenteric arterial (SMA) flow (i.e. portal flow) was reduced to multiple independent levels with stabilization periods between responses in order to produce variable degrees of buffer responses (HABR). The reductions were set to be at 20%, 40%, 60%, 80%, and 100% of control SMA flow by using the micrometer-controlled screw clamp. The sequence of the decrease in SMA flow was done randomly.

After obtaining graded multiple hepatic arterial buffer responses, intraportal adenosine infusion was started followed by full capacity HABR (i.e. 100% occlusion of SMA flow). These responses were considered as controls. Intrahepatic arterial MIX infusion was then started and the externally administered adenosine response and the full buffer capacity were repeated.

2.6 AUTOREGULATION OF HEPATIC ARTERIAL BLOOD FLOW

On three occasions, stepwise pressure-flow curves (similar to that seen in Figure 16) were compared with curves obtained by a series of independent pressure decreases with stabilization periods between responses (Figure 8). The curves appeared the same and so all tests used the more rapid and convenient method of stepwise pressure changes. Control responses were obtained to adenosine infusion, the hepatic arterial buffer response, and a pressure-flow curve. The 8-PT

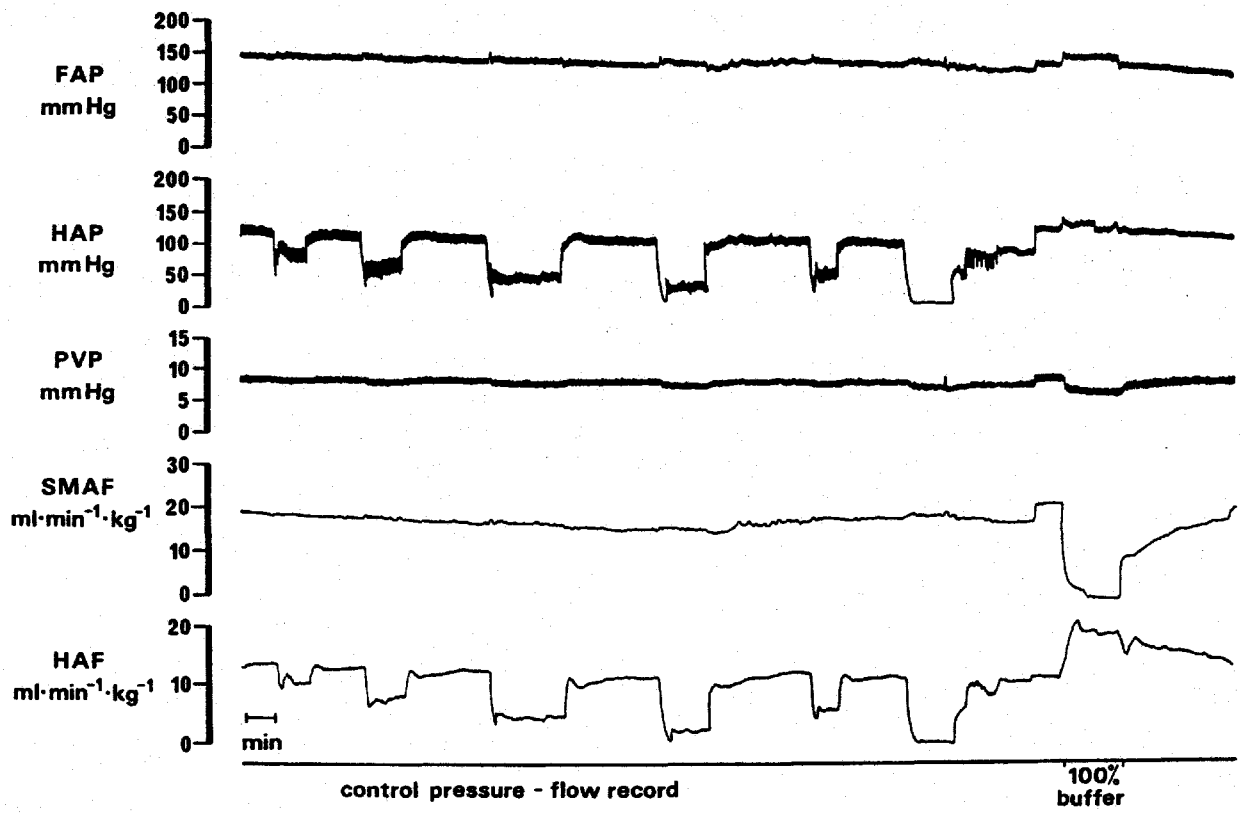

Fig. 8 Non-stepwise pressure-flow record with control base line intervals in between responses followed by control hepatic arterial buffer response during complete occlusion of superior mesenteric arterial flow (SMAF). Note that SMAF shows no reciprocal response to changes in hepatic arterial flow.

bolus dose was given in a progressively increasing fashion starting with 0.1mg.Kg^{-1} body weight to a maximum value of 16mg.Kg^{-1} body weight (n=5). The bolus dose was administered over one minute to be followed, after baseline stabilization, by adenosine infusion. Once the response to the externally administered adenosine was blocked, the stepwise reduction in the hepatic arterial pressure and occlusion of the portal flow (to induce the hepatic arterial buffer response) were performed.

In another group of cats (n=6) the systemic blood pressure was temporarily elevated, prior to the commencement of the experiment, by occluding the aorta below the kidneys. This allowed the pressure-flow curve to be obtained over a larger pressure range. This procedure was previously shown to produce elevation in arterial pressure with no effect on stroke volume, cardiac output or myocardial shortening (Stokland et al., 1981). In this group two kinds of adenosine receptor blockers were used, 8-PT (n=3) and 8-SPT (n=3). Pressure-flow curves and the buffer response were also determined during continuous norepinephrine infusion (n=5) at a rate of 0.1ug.min^{-1}.Kg^{-1} body weight or isoproterenol infusion (n=4) at a rate of 0.2ug.min^{-1}.Kg^{-1} body weight intraportally.

2.7 MATHEMATICAL AND STATISTICAL CALCULATIONS

Three means have been used to quantitate the hepatic arterial buffer response: the absolute rise in hepatic arterial conductance (HAC), the percent change in hepatic arterial conductance (% Δ HAC), and the buffering capacity. The buffering capacity was calculated from the absolute rise in hepatic arterial flow divided by the absolute decrease in SMA flow (i.e. portal flow). This value was expressed as percent where 100% would indicate a complete buffering of reduced portal flow by the hepatic artery.

Therefore:

$$\text{Hepatic Arterial Buffer Capacity} = \frac{\text{change in HAF}}{\text{opposite change in Portal (SMA) flow}} \times 100\%$$

The hepatic arterial autoregulation has been quantitated according to three indices. 1) The percent change in hepatic arterial resistance (% Δ HAR) over the autoregulatory pressure range. 2) The autoregulatory index (ARI) over the same autoregulatory range (Semple and DeWardener, 1959). The ARI was calculated according to this formula:

$$ARI = \frac{(HAF_C - HAF_S)/HAF_C}{(HAP_C - HAP_S)/HAP_C}$$

HAF and HAP are hepatic arterial flow and pressure; c signifies the control or initial state and s indicates values measured at the shoulder of the pressure-flow curve. It is evident from the ARI formula that ARI is equal to the percent change in hepatic arterial flow for each unit percent change in the hepatic arterial pressure. If a unit % change HAP is

accompanied by a unit % change HAF, then ARI value will be equal to one. ARI values of one or more indicate no active change in the vascular bed to autoregulate blood flow. On the contrary it means that the resistance of that vascular bed is either constant or decreasing with the increase in the pressure gradient across that vascular bed. ARI values of less than one mean an increase in resistance with the increase in pressure gradient across that vascular bed, which indicates the existence of pressure-flow autoregulation in that vascular bed. 3) A new index, the slope index, which is basically a test of linearity of the pressure-flow curve (see Figure 18 for description). In addition, where a calculation required use of a pressure gradient across the hepatic artery, we have calculated the arterial pressure gradient (in some experiments) in three ways: (a) absolute arterial pressure (equivalent to studies that assume zero hepatic venous pressure); (b) absolute arterial pressure minus sinusoidal pressure (portal venous pressure - see Discussion Section 4.6); (c) absolute arterial pressure minus critical closing pressure. For comparison of the various methods see the discussion.

Hepatic arterial resistance is calculated from arterial pressure gradient across the hepatic artery divided by arterial blood flow. i.e.

$$HAR = \frac{\text{HAP Gradient}}{\text{HAF}} \qquad \text{mmHg.ml}^{-1}\text{.min.Kg}$$

Unless mentioned otherwise, the gradient will be calculated as HAP minus sinusoidal (portal) pressure. Conductance, used in calculations too, is the inverse of resistance.

$$HAC = \frac{1}{HAR} = \frac{HAF}{HAP\ Gradient} \qquad ml.min^{-1}.Kg^{-1}.mmHg^{-1}$$

For the purpose of pooling different pressure-flow curves in the autoregulation series, the curves from each experiment were normalized. Normalization was essential since raw data were highly variable within and between experiments. The autoregulation series, as described above, was divided into raised and unraised pressure subseries. The HAF at HAP of 120mmHg (in the raised pressure subseries) and at HAP of 100mmHg (in the unraised pressure subseries) were chosen as control and were considered to be 100%. The remaining points on the curve were adjusted accordingly. Then the mean of the normalized HAF was obtained at 10mmHg intervals (Figure 17).

Comparison of various responses was made using paired t-test and two way analysis of variance. The paired t-test was used to determine whether the difference between two related data points was significantly different from zero. Both raw and normalized data were tested since each of the groups of related data points are believed to be normally distributed (i.e. the differences between paired observations are also normally distributed). Means of multiple response data points (such as raw or normalized data points on a curve) were compared by using two-way analysis of variance since all responses appeared once in each cat. The change was

considered significant when $p<0.05$. Changes in conductance or resistance were expressed as percent of basal level. Slopes of each hepatic arterial pressure-flow curve (above and below the shoulder - see Figure 18) were calculated from the raw data points by the use of regression analysis. Data were expressed throughout as means and standard errors.

3. RESULTS

3.1 THE QUANTITATION AND MECHANISM OF THE HEPATIC ARTERIAL BUFFER RESPONSE

3.1.1. The Quantitation of the HABR

To quantitate the HABR, the superior mesenteric arterial flow (which is equivalent to portal flow in our preparation) was reduced to 20, 40, 60, 80, and 100% of its control level using the micrometer screwclamp. The degree of reduction was calculated instantly depending on the flowmeter display recording. The method used to initiate variable degrees of HABR is clearly demonstrated in Figure 9. After each HABR the hepatic and superior mesenteric arterial flow were allowed to return to control levels to ensure validity of the results.

The hepatic arterial buffer response was quantitated by the use of three mathematical expressions: the response change in hepatic arterial flow (HAF) for each unit change in superior mesenteric arterial flow (SMAF) expressed as percent [(ΔHAF/ΔSMAF) x 100%], the percent change in hepatic arterial conductance (% Δ HAC) and the absolute change in HAC. The first method of calculation of the HABR capacity is more appropriate to quantitate the buffer response since it reflects the stimulus-response relationship between the superior mesenteric artery (SMA) and the hepatic artery (HA). The other two methods are less appropriate to express the capacity of the HABR as the SMAF is not included. The first method is used in this thesis to calculate the buffering capacity wherever required. The other two methods were used

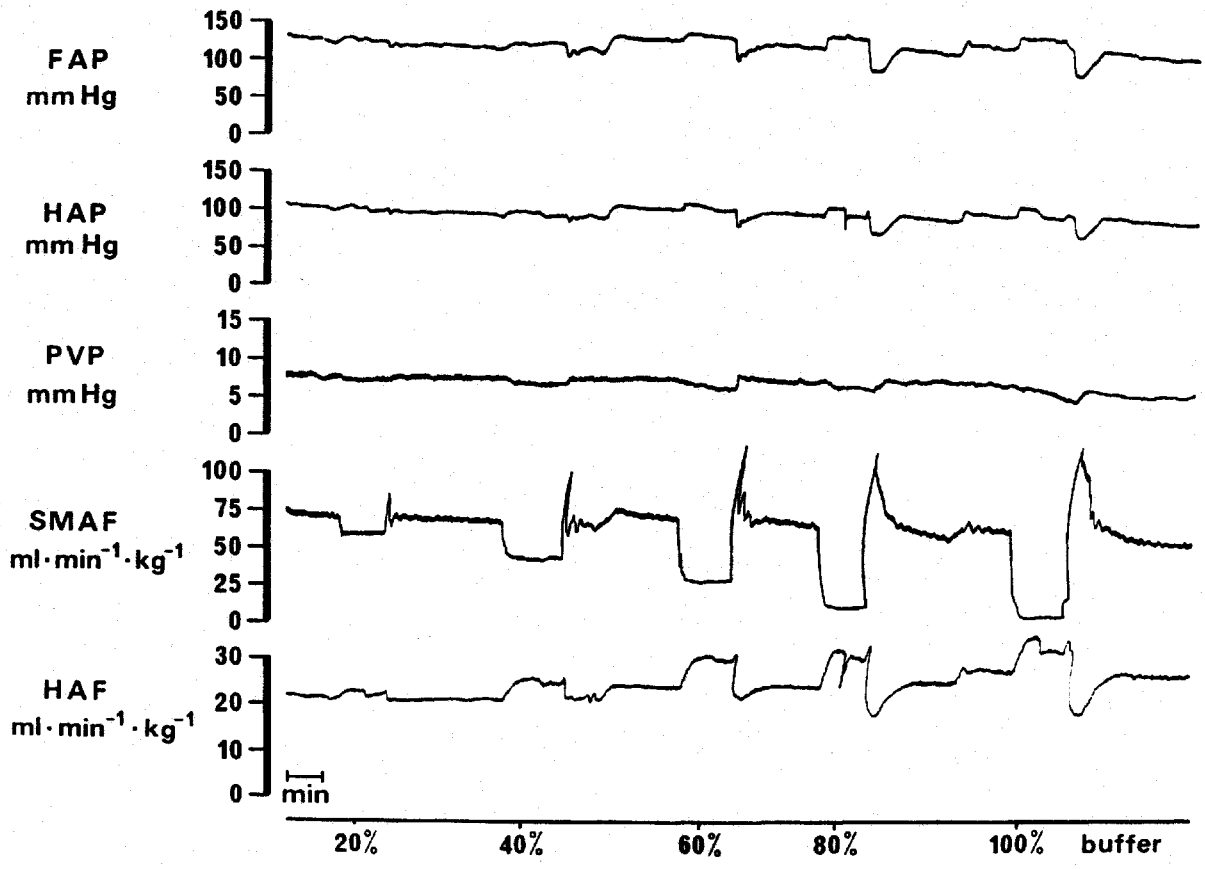

Fig. 9 Record of the relationship between hepatic arterial buffer response and stepwise decrease in superior mesenteric arterial blood flow.

to compare and confirm results obtained by the application of the first method.

The relationship between the capacity of HABR and the changes in SMAF is shown in Figure 10. The capacity of the HABR increased with the decrease in SMAF to reach its highest level of 24.24 ± 2.86 at 60% decrease in SMAF. Further decrease in SMAF did not increase the buffering capacity, it rather decreased it. The buffering capacity at 100% decrease in SMAF (17.69 ± 3.45) was not significantly different from that of 20% reduction in SMAF (15.14 ± 2.66). However, the peak buffering capacity obtained at 60% decrease in SMAF was not statistically significantly different from that reached at 40 and 80% decrease in SMAF.

The calculated buffering capacity mentioned above was obtained after the correction of the hepatic arterial pressure (HAP) to be equal to its control value prior to the occlusion of the superior mesenteric artery (Refer to Figure 3, stage C). However, in normal physiological situations hepatic arterial flow changes coexist with changes in hepatic arterial pressure as well, i.e. the HAP is not constant. Therefore, the data were recalculated to obtain the buffering capacity of the hepatic artery during uncontrolled HAP (Figure 3, stage B). The HABR capacity under uncontrolled HAP was found to be constant at any degree of decrease in SMAF with a mean value of 30.30 ± 2.79. The comparison between the buffering capacities of the HAP-controlled and uncontrolled situations

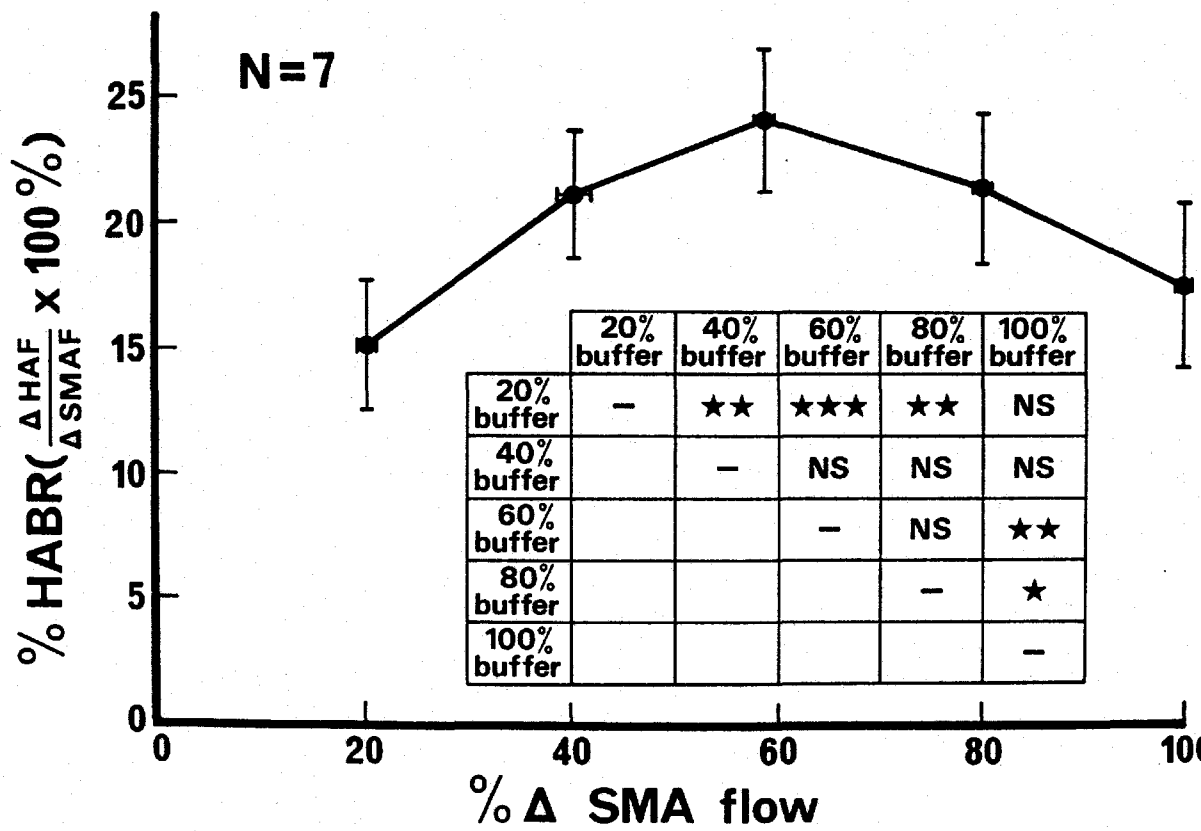

Fig. 10 The relationship between the hepatic arterial buffer capacity and superior mesenteric arterial (SMA) flow (Mean±SE). HABR = hepatic arterial buffer response, %△ = percent change, Δ HAF = change in hepatic arterial flow, Δ SMA = change in superior mesenteric arterial flow (= portal flow).

are clearly illustrated in Figure 11. The HAP-uncontrolled data showed a tendency for the buffering capacity to decline slightly after 60% decrease in SMAF, however, that decrease was not significant statistically.

To quantitate the buffering capacity of the HABR using a different criterion, the percent change in hepatic arterial conductance (% Δ HAC) was used (Figure 12). The change in hepatic arterial conductance increased sharply from a value of 4.80 ± 0.96% (at 20% decrease in SMAF) to a value of 23.01 ± 1.86% (at 60% decrease in SMAF), then reached a maximum value of 28.68 ± 3.59% at 100% decrease in SMAF (i.e. complete occlusion of the SMA). The %ΔHAC reached at 60% decrease in SMAF was significantly different ($P<0.01$) from that reached at 100% reduction, but was not different from that reached at 80% reduction in SMAF. The %Δ HAC values attained at 80% and 100% decrease in SMAF were not significantly different from each other.

To demonstrate the effect of HAP on %ΔHAC, the data were recalculated to obtain HAC during uncorrected HAP. The hepatic artery showed some degree of autoregulation since HAC at higher HAP was less than that at lower HAP. In other words, HAC calculated during phase C (Figure 3) was higher than that calculated from phase B. The maximum difference between HAP-controlled and uncontrolled %Δ HAC was reached at 80% decrease in SMAF (HAP-controlled %Δ HAC = 25.97 ± 2.31%; HAP-uncontrolled %ΔHAC = 20.29 ± 2.21%). The difference was

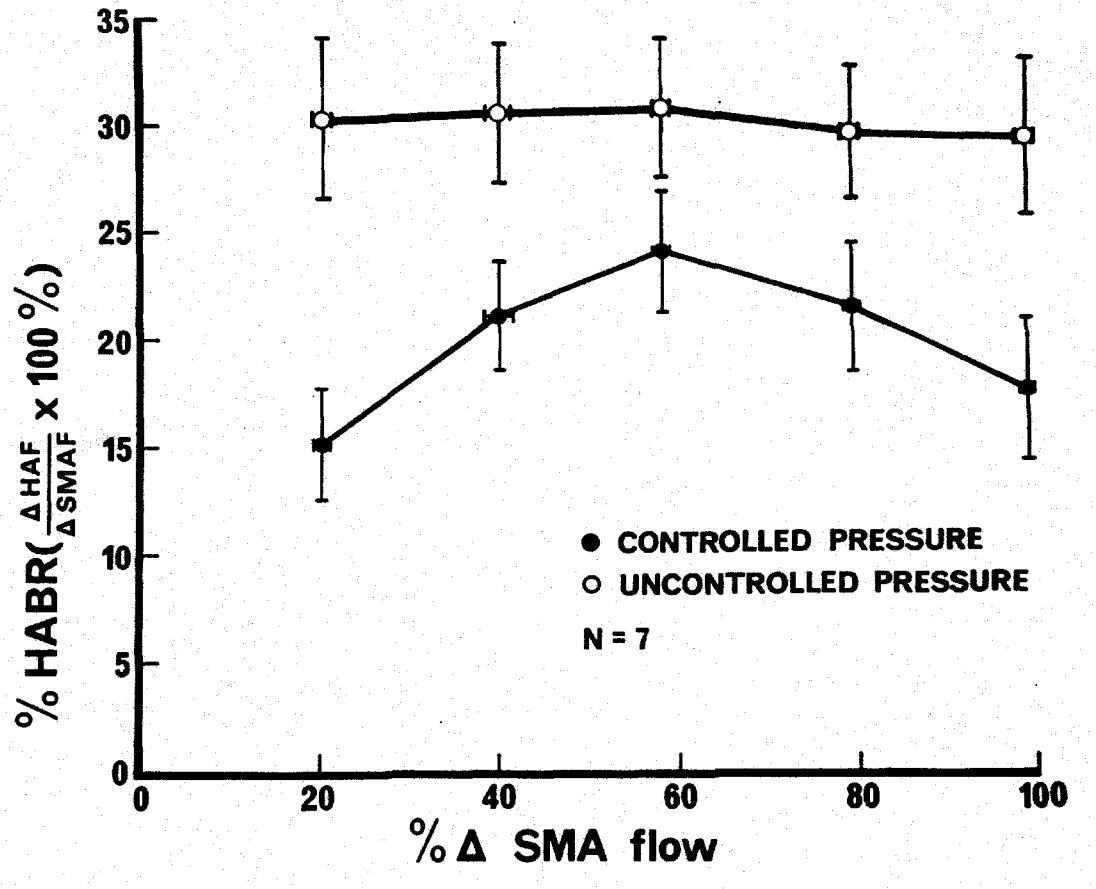

Fig. 11 The relationship between the buffer capacity and superior mesenteric arterial flow during controlled and uncontrolled hepatic arterial pressure (HAP). The buffer capacity remains stable when HAP was left uncontrolled.

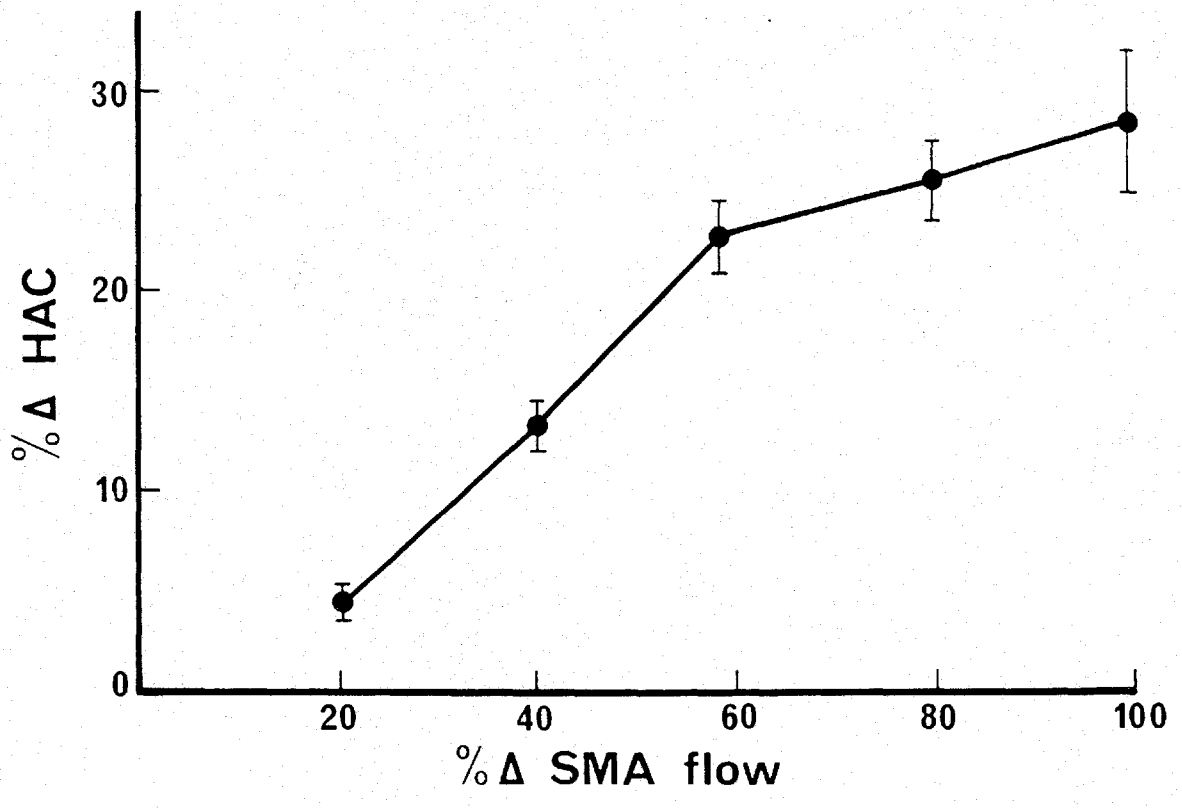

Fig. 12 Percent change in hepatic arterial conductance (%△HAC) during stepwise decrease in superior mesenteric arterial (SMA) flow (Mean ± SE).

highly significant statistically ($P<0.01$). At complete occlusion of the SMA the difference decreased and became not significant statistically.

The actual change in HAC was used as a third criterion to quantitate the buffering capacity of the HABR. The basal conductance of the hepatic artery in the quantitation series was 0.196 ± 0.013 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ (35 observations). The HAC increased steadily, with the decrease in SMAF, to a peak value of 0.254 ± 0.043 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ at 80% decrease in SMAF (Figure 13). However, HAC peak values reached at 60, 80 and 100% decrease in SMAF were not significantly different from each other. Again the comparison between HAC during HAP-controlled and uncontrolled phases revealed a maximum difference at 80% drop in SMAF, however, the difference was not statistically significant.

To test whether the highest HABR capacity was because of full dilation of the hepatic artery, a paired-comparison was made between the highest (peak) HAC reached during HABR and the HAC during the intraportal venous infusion of adenosine ($0.4 - 1 mg.Kg^{-1}.min^{-1}$) ($N=7$). Adenosine infusion dilated the HA and elevated HAC to a mean value of 0.299 ± 0.045 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ which is significantly higher ($P<0.05$) than that (0.240 ± 0.042 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$) reached during peak buffer. Therefore, it was concluded that peak HABR was not because of full dilation of the hepatic artery since it can

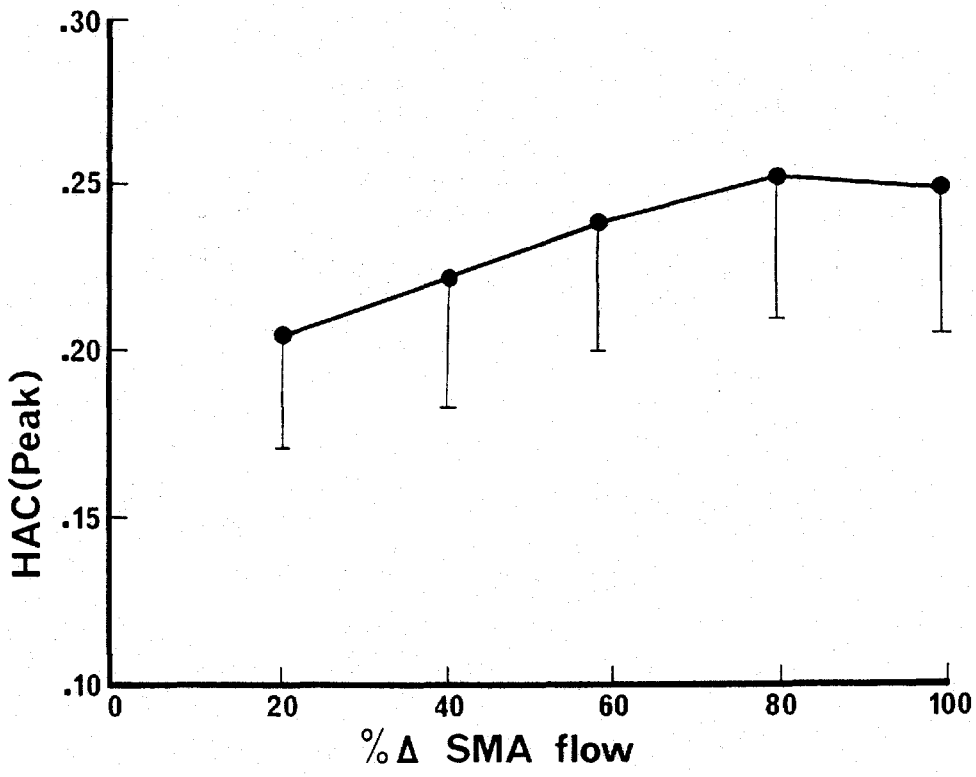

Fig. 13 The change in absolute peak hepatic arterial conductance (HAC) during stepwise decrease in superior mesenteric arterial (SMA) flow (Mean ± SE).

dilate more in response to the infusion of a vasodilator such as adenosine.

3.1.2 The Mechanism of the HABR

3.1.2.1 Effect of MIX on Systemic and Hepatic Hemodynamics

The adenosine receptor blocker MIX (1-Methyl-3-isobutyl-xanthine) was infused at a rate of 0.1 $mg.min^{-1}.Kg^{-1}$ directly into the hepatic artery in a base carrier (N=5). Its infusion caused significant local and systemic hemodynamic changes. Systemic blood pressure (BP) dropped from a control value of 122.3 $\pm$ 7.4 to 74.6 $\pm$ 6.7 mmHg ($P<0.01$). The hepatic arterial conductance increased significantly from a control value of 0.183 $\pm$ 0.041 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ prior to MIX infusion to a mean value of 0.364 $\pm$ 0.086 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ during MIX infusion ($P<0.01$). This increase in HAC was found to be equal to 86.99 $\pm$ 15.21% of its control value. In other words, MIX nearly doubled the basal HAC. Total liver blood flow did not change significantly from a control value of 53.98 $\pm$ 9.05 to 55.61 $\pm$ 12.59 ($ml.min^{-1}.Kg^{-1}$) during MIX infusion. Portal venous pressure (PVP) decreased slightly during MIX infusion from a control value of 8.7 $\pm$ 0.4 mmHg to 8.2 $\pm$ 0.2 mmHg. This decrease was statistically insignificant.

3.1.2.2 Effect of MIX on HABR

Adenosine was found to be a potent dilator of the hepatic artery. The dilatory response is nearly immediate and shows no tachyphylaxis. A testing dose of 0.4$mg.Kg^{-1}.min^{-1}$

intraportally was able to increase the HAC by 72.67 ± 11.48%. Higher doses produce more dilation but can show some systemic effect as well. Intrahepatic arterial infusion of MIX (0.1 $mg.Kg^{-1}.min^{-1}$) significantly inhibited the dilating response to the externally administered adenosine as well as the HABR (Figure 14). In this figure the HABR was quantitated as percent change in HAC. The same result was obtained when the buffering capacity was calculated. MIX reduced the buffering capacity from 24.88 ± 3.95% to a lower value of 9.73 ± 2.78% (N=5). This inhibition was highly significant ($P<0.01$).

The above mentioned data make it clear that MIX is a non-specific adenosine receptor blocker. Other than its receptor blocking property, it induces many significant hemodynamic changes namely its prominent dilation of the hepatic artery. It can be argued at this stage that the failure of the hepatic artery to dilate in response to infused adenosine or reduced SMAF can be attributed to its maximal dilation induced by MIX infusion. This possibility is quite valid since the maximal HAC reached during HABR during MIX infusion (0.401 ± 0.090 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$) was not significantly different from the peak HAC (0.422 ± 0.085 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$) reached during adenosine infusion during the same stage. Nevertheless, this statistical comparison cannot differentiate between the blocking selectivity of MIX and its vasodilation effect. The

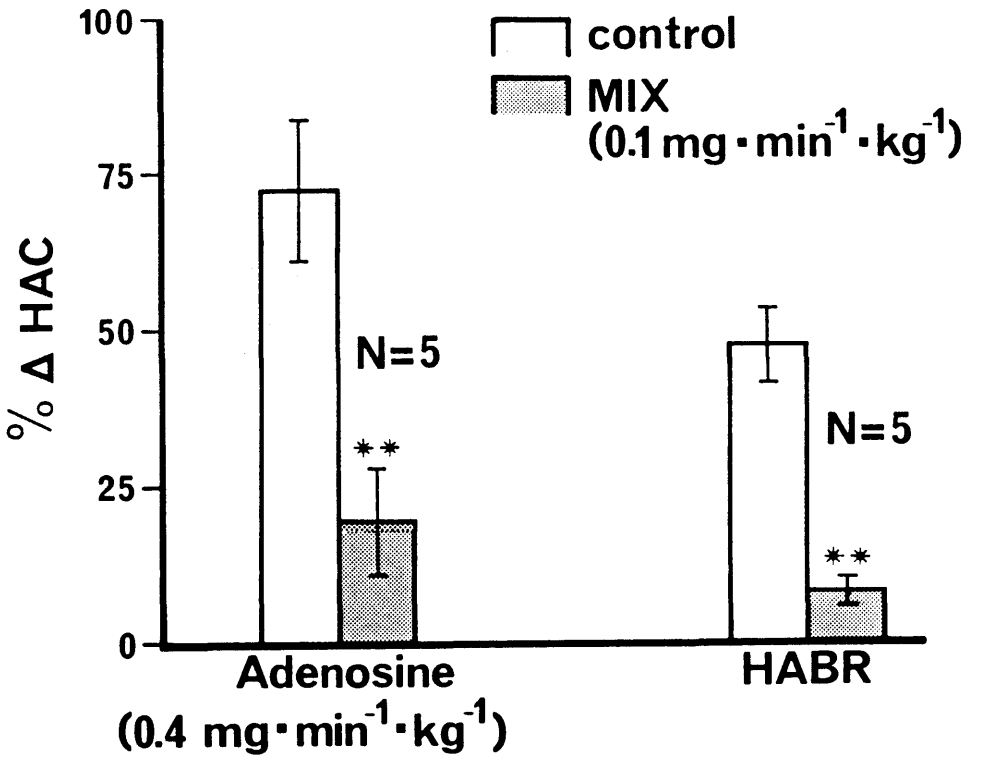

Fig. 14 Effect of MIX on the percent change in hepatic arterial conductance (%△HAC) during externally administered adenosine and hepatic arterial buffer response (HABR).

hepatic artery under MIX effect should be tested with another dilator, other than adenosine, to prove or disprove that it was maximally dilated and that it could still react to other vasoactive substances. Since such a test was not conducted in this series, the data cannot exclude the possibility that the hepatic artery was maximally or submaximally dilated. However, there was more than one reason to suggest that the inhibition of the HABR and the dilator response to infused adenosine were related to the blockade of adenosine receptors. The discussion of these reasons will be presented later. The non-selective blockade property of MIX makes it essential to use other more selective adenosine receptor blockers that do not affect the basal conductance of the hepatic artery. The adenosine receptor blockers 8-PT and 8-SPT were used, for that reason, in the subsequent experiments.

MIX was found to be a labile adenosine receptor blocker. Responses to infused adenosine return to normal within 5-10 minutes after cessation of the infusion of MIX despite the well maintained systemic effects.

MIX seems to counteract the effect of anesthesia. The anesthetic level of the animal wears off during MIX infusion. This effect was manifested as recurrence of brisk swallowing and corneal reflex. The respiration rate increased and became shallow with periodic gasps. Spontaneous flickering of the ears was not uncommon. On the other hand, it was inconvenient to give the animal supplementary doses of anesthesia because

of its lower blood pressure due to the MIX-induced systemic vasodilation. Therefore, MIX is not a convenient adenosine blocker to be used in the whole animal preparation.

3.2 PRESSURE-FLOW AUTOREGULATION OF THE HEPATIC ARTERY

Any cat that did not show dilation of the hepatic artery to infused adenosine was excluded from this study. A response to adenosine and a functional buffer response were used to assess pharmacological blockade of adenosine receptors. Previous unpublished observations, by us, were that if the hepatic artery does not respond to adenosine, it does not show dilation to reduced portal flow (i.e. HABR). In this series, about 50% of cats were eliminated from this study according to this inclusion criterion, because of lack of response to the test dose of adenosine. Every cat that showed a response to adenosine also showed a buffer response and hepatic arterial autoregulation. Three cats that did not show response to adenosine were tested and found not to show autoregulation; no others were similarly tested.

One group of cats was studied under normal blood pressure conditions (unraised-pressure series, N=5), while a second group was studied in the same way using aortic occlusion below the kidneys to elevate systemic pressure (raised-pressure series, N=6). The evaluation and comparison of the capacity of autoregulation, according to the three indices used, was entirely based on absolute data. However, visual comparison

between a group of pressure-flow curves cannot be done without standardization of these curves. Curves vary in the degree of curvature, the position of the curve on the pressure axis, the location of the shoulder and the initial flow varied from curve to curve within the same experiment and between the experiments. To standardize a group of curves, the highest shared arterial pressure point is determined. The hepatic arterial flow of each curve, at that common pressure point, is considered as 100% for that particular curve. The rest of the flow points on the curve will be expressed as percent of the 100% (control) point of that curve. In the unraised pressure group, pressure-flow curves were standardized from a control blood pressure of 100mmHg; the raised pressure group was standardized from 120mmHg. Each individual pressure-flow curve was sigmoid in shape and concave to the pressure axis (control curve of Figure 15). However, the process of standardization and pooling of different curves underestimated the degree of concavity of each individual curve.

To obtain a pressure-flow curve, HAP was reduced in stepwise fashion by the use of the micrometer-controlled screwclamp around the hepatic artery as mentioned above (in the methods). In the raised-pressure group the stepwise reduction of HAP was preceded by the occlusion of the aorta below the kidney to raise the systemic blood pressure. Figure 16 shows both the pressure-flow responses and effect of aortic occlusion on HAP in one cat from the raised pressure series.

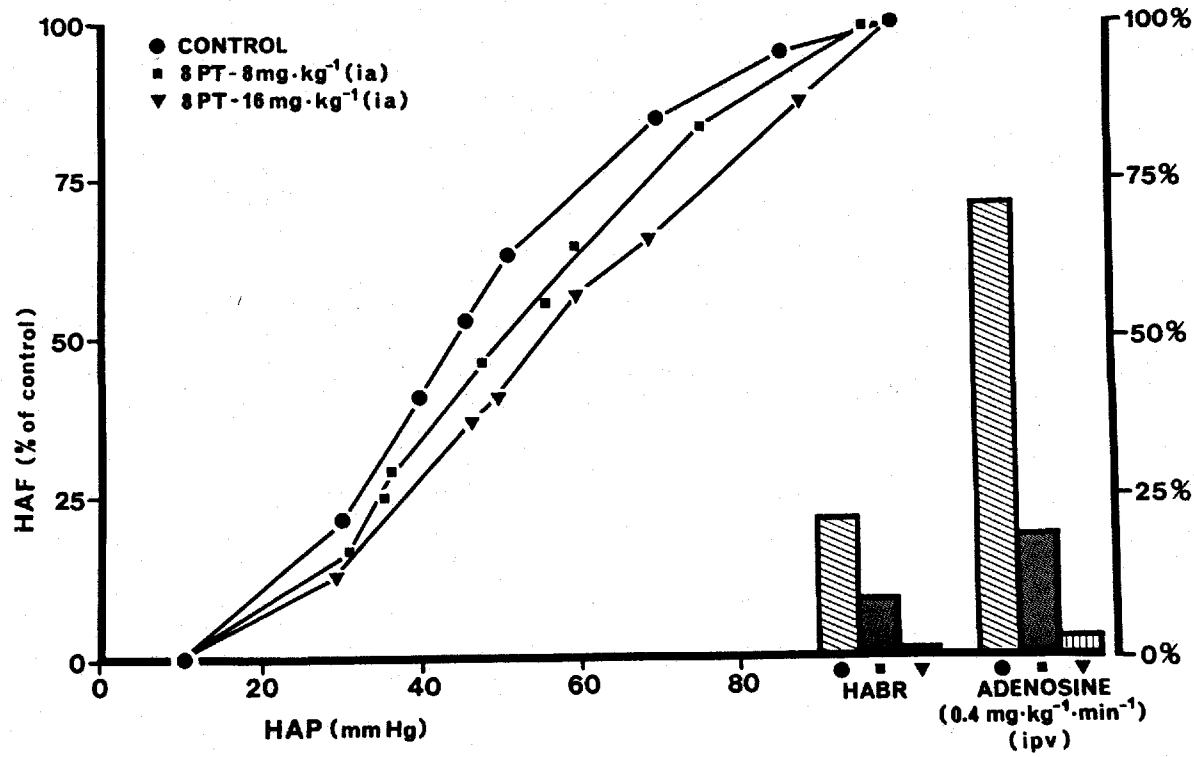

Fig. 15 Relationship of hepatic arterial perfusion pressure (HAP) to hepatic arterial flow (HAF) presented as percent of control flow, before and after administration of two bolus doses of adenosine blocker in one cat. Histograms represent effect of adenosine blocker on hepatic arterial buffer response (HABR) expressed as rise in HAF divided by decrease in portal flow (x100%) and adenosine response expressed as percent rise in vascular conductance. Note that the first dose produced partial antagonism of the HABR, the dilator effect of exogenous adenosine and autoregulation.

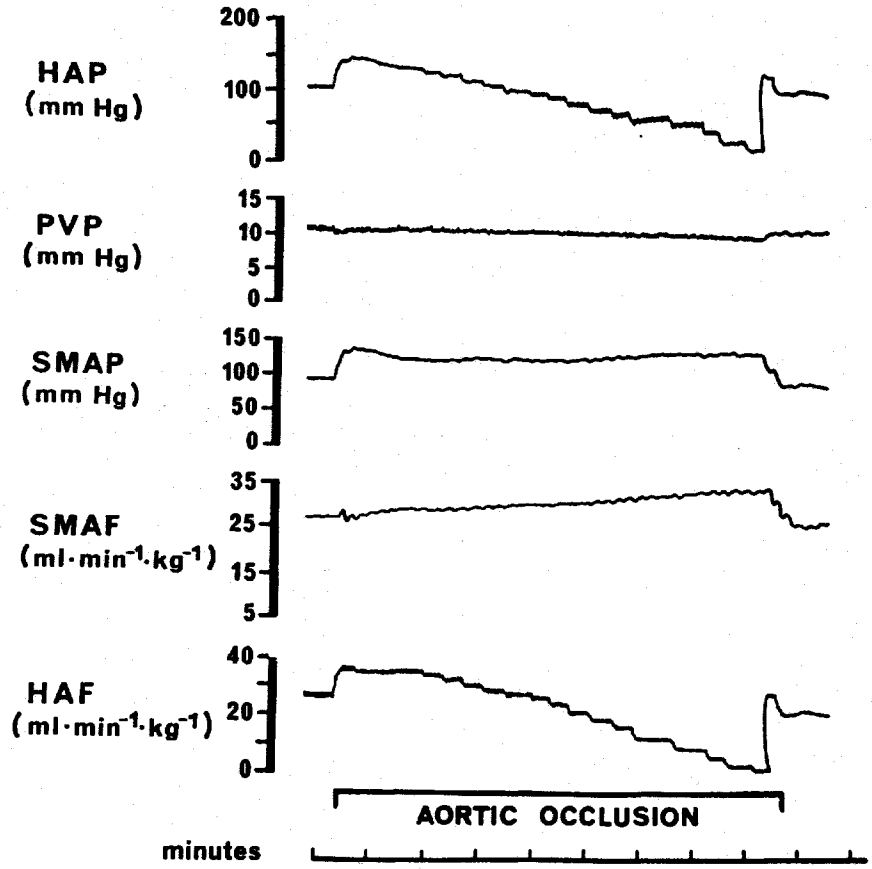

Fig. 16 Trace taken from one cat to illustrate effect of aortic occlusion on splanchnic hemodynamics followed by stepwise reduction in hepatic arterial pressure (HAP) and flow (HAF). PVP, portal venous pressure; SMAP, superior mesenteric arterial pressure; SMAF, superior mesenteric arterial flow.

The mean rise in systemic blood pressure after lower aortic occlusion was similar in the control state (13.1 ± 4.0mmHg) and in the adenosine receptor blocked state (13.8 ± 3.5mmHg). The stepwise reduction in hepatic arterial pressure and flow was accompanied by a small rise in flow (3.5 ± 1.2ml/min per Kg) of the superior mesenteric artery (portal flow) which was similar in the adenosine blocked state (3.3 ± 1.3ml/min per Kg). The aortic occlusion tended to cause mild constriction (Table 1) but did not cause statistically significant change in basal conductance of the superior mesenteric artery or the hepatic artery or in the critical closing pressure in the control or blocked state. The mean critical closing pressure (ccp) pooled from both groups was 11.5 ± 1.4mmHg and portal pressure was 8.5 ± 0.6mmHg, significantly lower ($P<0.02$) than ccp. The raised-pressure group was initiated in order to provide a longer pressure-flow curve.

3.2.1 Assessment of Adenosine Antagonism

The bolus blocking dose range used for 8-PT was 0.8-16mg.Kg^{-1} body weight and 4 - 16mg.Kg^{-1} body weight for 8-SPT. The adenosine receptor blocker 8-PT was more potent and more stable compared to 8-SPT. The blocking property of 8-PT could be detected at a low dose of 0.4mg.Kg^{-1}. A supermaximal dose of 16mg.Kg^{-1} caused the hepatic artery to constrict and HAF to drop to zero. The blocking effect of 8-PT was reversible and lasted about 20-30 minutes. The water soluble adenosine receptor blocker 8-SPT started to show its blocking property

at a bolus dose of 2mg.Kg^{-1} in some preparations (range 4-8mg/Kg). In others it failed to show any blocking property even at a dose of 16mg.Kg^{-1}, and therefore, it was substituted by 8-PT. The blocking property of 8-SPT was reversible as well and lasted for about 15-20 minutes. The two blockers did not alter basal parameters as shown in Table 1. However, 8-PT showed a tendency to vasoconstrict the hepatic artery. This hepatic arterial vasoconstriction was not significant statistically. The blocker 8-SPT seemed to have no vasoactive property. The unraised pressure group received only 8-PT (N=5). The blockers 8-SPT (n=3) and 8-PT (n=3) were used in the raised pressure series and the data were pooled.

The adenosine antagonists 8-PT and 8-SPT blocked the hepatic arterial vasodilator response to intraportal infusion of adenosine (Table 2) in both groups. Similarly, the hepatic arterial buffer response was significantly antagonized. Note that after testing these responses following blockade of adenosine receptors, a final dose of adenosine was given to assure that blockade had not worn off since we commonly see potentiation of adenosine response immediately following recovery from blockade. Lautt and Legare (1985) tested the effect of 8-PT administration on isoproterenol. The vasodilator response to isoproterenol infusion was maintained, and therefore, it was concluded that the blockade was selective for adenosine and did not represent a deterioration of the preparation.

TABLE 1 Values are means and standard errors measured before and after administration of a blocking dose of 8-PT (n=3) or 8-SPT (n=3) in the group with raised blood pressure and 8-PT in the group with unraised pressure. HAF is hepatic arterial flow; HAR is HA resistance; HAC is conductance; ccp is critical closing pressure; PVP is portal venous pressure. HAR and HAC are calculated using zero venous pressure to allow comparison with previous autoregulation studies. Recalculated HAR and HAC using the recommended procedure of HAP - PVP to calculate perfusion pressure showed no change in these or other statistical tests except that where a change from control to blocked state was significant, the level of significance was greater using HAP - PVP rather than HAP-zero (see Table 2). No basal parameters were significantly altered by the adenosine antagonists (paried-t test, n=6).

TABLE 1

EFFECT OF ADENOSINE BLOCKERS ON BASAL PARAMETERS

RAISED PRESSURE GROUP
(N=6; HAP=120 mmHg)

	CONTROL	ADENOSINE BLOCKER
HAF ($ml.min^{-1}.Kg^{-1}$)	27.1 ± 9.1	33.7 ± 12.2
HAF as % of total liver flow	42.7 ± 3.7	47.6 ± 5.7
HAR ($mmHg.ml^{-1}.min.Kg$)	6.5 ± 1.4	5.6 ± 1.3
HAC ($ml.min^{-1}.Kg^{-1}.mmHg^{-1}$)	0.23 ± 0.08	0.28 ± 0.10
ccp (mmHg)	13.6 ± 1.9	14.6 ± 1.9
PVP (mmHg)	9.0 ± 0.9	9.8 ± 0.6

UNRAISED PRESSURE GROUP
(N=5; HAP=100 mmHg)

	CONTROL	ADENOSINE BLOCKER
HAF ($ml.min^{-1}.Kg^{-1}$)	37.3 ± 11.7	22.3 ± 5.0
HAF as % of total liver flow	38.0 ± 7.0	30.3 ± 5.3
HAR ($mmHg.ml^{-1}.min.Kg$)	4.3 ± 1.6	5.7 ± 1.4
HAC ($ml.min^{-1}.Kg^{-1}.mmHg^{-1}$)	0.37 ± 0.12	0.22 ± 0.05
ccp (mmHg)	9.5 ± 1.3	11.3 ± 1.1
PVP (mmHg)	7.8 ± 0.4	8.6 ± 0.4

TABLE 2 Effect of adenosine blockers in the unraised and raised pressure groups on the response to infused adenosine, on an intrinsic response previously shown to be adenosine mediated (HABR) and on indices of autoregulation. Effects of adenosine infusion (0.4 mg/Kg per min, i.p.v.) are shown as percent change in conductance and resistance. The hepatic arterial buffer response (HABR) capacity is expressed as the increase in arterial flow divided by the reduction in portal flow as a percent. Indices of autoregulation are: ARI, autoregulatory index calculated according to (Semple and DeWardener, 1959) where zero venous pressure is assumed; % change in hepatic arterial resistance seen when blood pressure is decreased from control state (120 mmHg in raised pressure group, 100 mmHg in unraised pressure group) to shoulder of pressure-flow curve (70 $\pm$ 6 mmHg); slope index is the slope of the pressure-flow curve over the autoregulatory range divided by the slope below the autoregulatory range (see Figure 18). All responses were significantly impaired by adenosine blockers (paired-t test; * = $p<0.05$; ** = $p<0.01$).

TABLE 2

EFFECT OF ADENOSINE BLOCKERS ON INTRINSIC REGULATION OF THE HEPATIC ARTERY

		CONTROL	ADENOSINE BLOCKER	P
RESPONSE TO EXOGENOUS ADENOSINE:				
(%ΔHAC)	UNRAISED PRESSURE GROUP	41.5 ± 9.0	5.8 ± 2.1	**
	RAISED PRESSURE GROUP	57.0 ± 15.4	5.5 ± 1.8	**
RESPONSE TO EXOGENOUS ADENOSINE:				
(%ΔHAR)	UNRAISED PRESSURE GROUP	-27.8 ± 3.8	-5.3 ± 1.9	**
	RAISED PRESSURE GROUP	-33.3 ± 5.8	-5.3 ± 1.7	**
RESPONSE MEDIATED BY ENDOGENOUS ADENOSINE:				
(HABR)	UNRAISED PRESSURE GROUP	27.7 ± 5.7	6.4 ± 3.7	**
	RAISED PRESSURE GROUP	28.5 ± 7.4	9.3 ± 3.1	*
CALCULATED INDICES OF AUTOREGULATION:				
ARI	UNRAISED PRESSURE GROUP	0.86 ± 0.06	1.11 ± 0.06	*
	RAISED PRESSURE GROUP	0.88 ± 0.05	1.19 ± 0.04	**
%ΔHAR	UNRAISED PRESSURE GROUP	-12.8 ± 4.4	0.2 ± 3.8	*
	RAISED PRESSURE GROUP	-14.4 ± 2.0	-1.1 ± 2.8	**
SLOPE INDEX				
	UNRAISED PRESSURE GROUP	39.6 ± 6.3	73.7 ± 7.8	*
	RAISED PRESSURE GROUP	53.7 ± 5.6	81.8 ± 5.3	**

3.2.2 Antagonism of Autoregulation

Figure 15 shows pressure-flow curves from one cat, where tests were done after partial and full blockade, standardized by expressing blood flow at 100mmHg pressure as 100%. The control curve shows the classic autoregulatory sigmoid curve, concave to the pressure axis, intercepting the pressure axis at a critical closing pressure above zero. The hepatic arterial buffer response (HABR) is shown in the histogram as the rise in arterial flow divided by the decrease in portal flow as a percent (i.e. the buffering capacity). The response to adenosine infusion is expressed as the percent rise in vascular conductance. The low dose of 8-PT decreased the HABR, the response to adenosine and also reduced hepatic arterial autoregulation (the curve becomes more linear). The higher blocking dose of 8-PT produced further depression of all three responses. The dose-related blockade of the HABR and response to exogenous adenosine is in support of the previously presented data of the quantitation and mechanism of HABR series (where MIX was used) and is also seen for autoregulation.

Figure 17 shows pooled results (1 control and 1 blocked response per cat) from the raised pressure group using blockade by 8-PT (n=3) and 8-SPT (n=3). All responses were antagonized. Note, however, that pooling the pressure-flow curves tends to conceal the sigmoid shape of each individual curve and presents a mean curve that implies an extremely weak

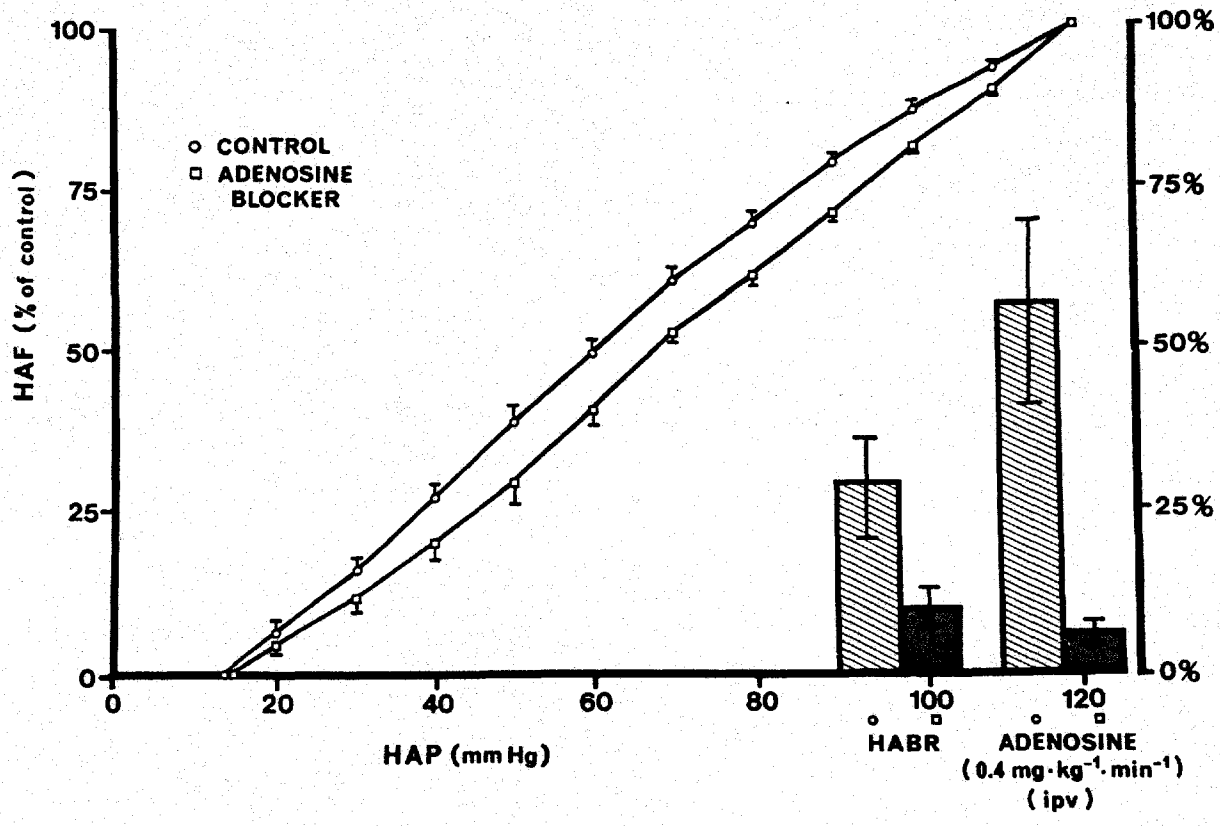

Fig. 17 Pooled relationship (n=6) of hepatic arterial pressure (HAP) to hepatic arterial flow (HAF) presented as percent of control flow, before and after bolus administration of adenosine blocker (8-PT, n=3; 8-SPT, n=3). Effect of adenosine blockers on hepatic arterial buffer response (HABR) and adenosine response is represented by histograms. HABR is expressed as rise in HAF divided by fall in portal flow (x 100%) elicited by complete occlusion of portal flow. Adenosine response is expressed as percent rise in vascular conductance. Blood pressure was raised by aortic occlusion.

autoregulation that is difficult to quantitate for statistical comparison with the curve obtained in the blocked state.

Assessment of linearity of the curve is one index of the presence of autoregulation. We used a new index, the autoregulatory slope index, as a means of assessing linearity of the curve. This index is based on the observation that over the autoregulatory range the slope of the curve is less than that seen below the autoregulatory range. The point at which the slopes deviate is the pressure at the low end of the autoregulatory range and is referred to as the "shoulder". Figure 18 shows these points on a hypothetical pressure-flow curve. As the slope index (slope over autoregulatory range divided by slope below autoregulatory range expressed as percent) approaches 100%, autoregulation becomes blocked. Table 2 shows significant blockade of autoregulation according to this index.

The autoregulatory index (ARI) proposed by Semple and DeWardener (1959) uses an approach that can avoid use of multiple points on a curve. Pressures and flows are compared in the basal state at some standardized pressure (100mmHg in our unraised pressure group; 120mmHg in the raised pressure group) and at a lower pressure representing the point of the shoulder on the pressure-flow curve (this point corresponded to the pressure where resistance was the lowest on the pressure-resistance curve). This index is a measure of the proportional change in flow divided by the corresponding

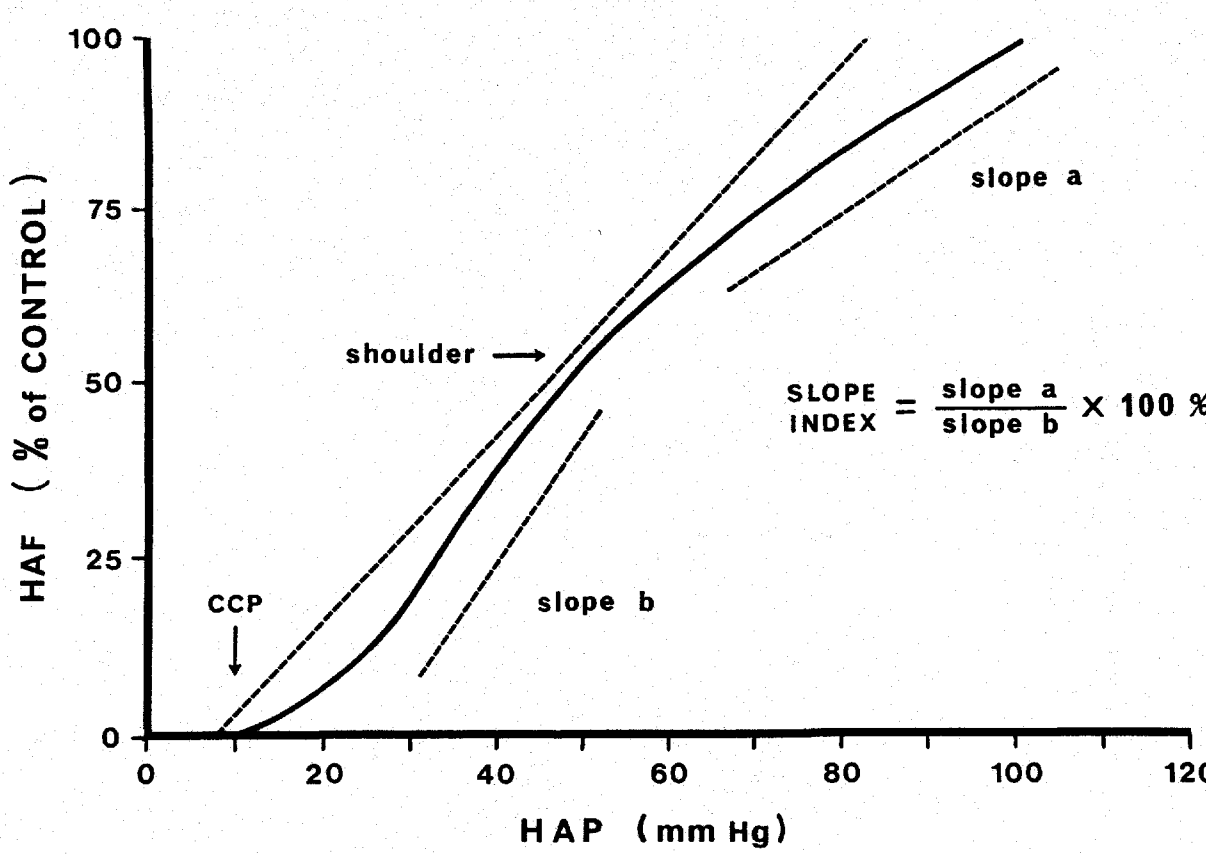

Fig. 18 Hypothetical pressure-flow curve showing autoregulation. Shoulder corresponds to point of minimal resistance on a pressure-resistance curve. Calculation of slope index as an index of linearity of the curve above (autoregulatory range) and below the shoulder is shown. Slope below the shoulder is taken over the linear portion above the tail near the critical closing pressure. Regression analysis over these ranges can compensate for slight non-linearity. Slope index of 100% indicates no differential change in resistance along the curve and no autoregulation. This index does not depend on calculation of vascular resistance with its inherent limitations.

proportional change in pressure. The autoregulatory index was significantly altered by the adenosine antagonists in both groups (Table 2). The mean control ARI (raised pressure group) using uncorrected pressure gradient was 0.882 ± 0.048, 0.823 ± 0.045 when portal pressure was subtracted and 0.782 ± 0.046 when critical closing pressure was subtracted from HAP. All ARI were significantly raised by adenosine antagonists.

Autoregulation implies that arterial resistance changes in response to altered perfusion. Mean resistance decreased progressively as pressure dropped to 70 ± 6mmHg (range 50-90mmHg) (the shoulder of Figure 18) while at lower pressure the resistance rose. To assess the existence of statistically significant hepatic arterial autoregulation, the difference between control hepatic arterial resistance (HAR) (at HAP of 120mmHg in the raised-pressure series) and HAR at the shoulder was tested. The change in HAR was significant whether portal venous pressure ($P<0.05$) or critical closing pressure ($P<0.01$) was used to correct for the pressure gradient. However, the change was not significant when uncorrected pressure gradient was used. There was no statistically significant difference between portal venous pressure and critical closing pressure values. Figure 19 shows mean data from the raised pressure group, standardized from basal resistance at arterial pressure of 120mmHg and expressed as a percent of basal resistance at 10mmHg pressure decrements over the autoregulatory range from individual curves. These data show the clearest graphic

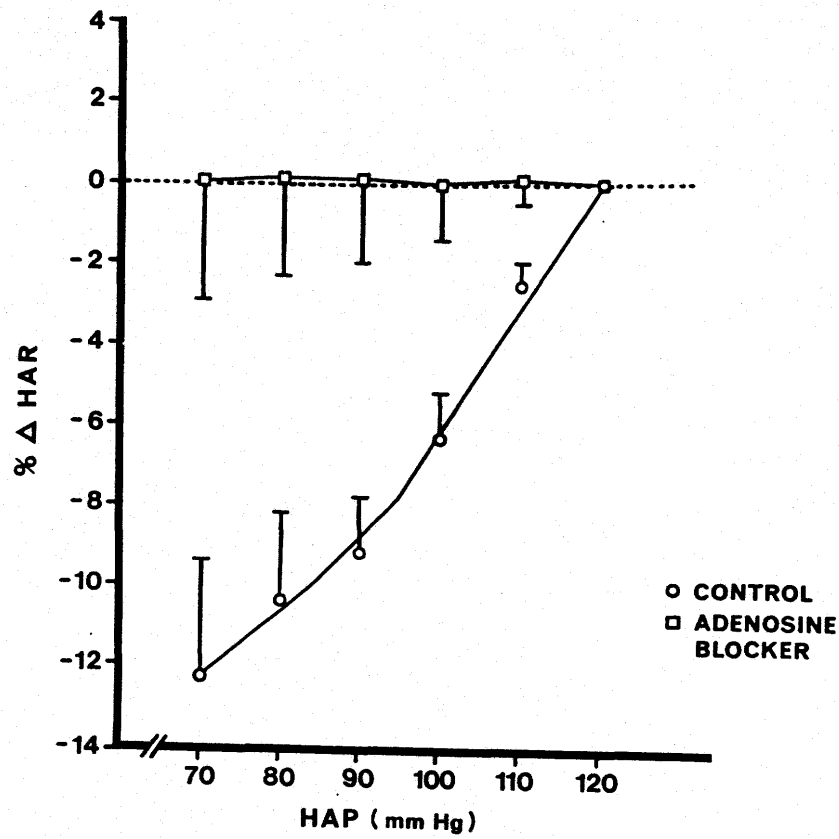

Fig. 19 Relationship of percent change in hepatic arterial resistance (% Δ HAR) to hepatic arterial perfusion pressure (HAP) over the autoregulatory range, before and after adenosine blocker bolus administration (n=6). Same group of animals as reported in Table 1 and 2, and Figure 17.

demonstration of autoregulation and blockade by adenosine receptor blockers. Table 2 shows the calculated percent change in resistance for both groups and clearly indicates that change in hepatic arterial resistance is prevented by the adenosine antagonists.

3.2.3 Effect of Norepinephrine and Isoproterenol Infusion on Hepatic Arterial Pressure-Flow Curve

Norepinephrine, when infused intraportally ($0.1ug.Kg^{-1}.min^{-1}$), significantly decreased hepatic arterial basal conductance (n=5) from 0.156 ± 0.020 to 0.102 ± 0.015 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ ($P<0.01$). Norepinephrine infusion significantly increased the hepatic arterial critical closing pressure from 17.8 ± 1.38 mmHg to 23.4 ± 1.87 mmHg ($P<0.01$) as well. Norepinephrine infusion induced no statistically significant changes on the autoregulatory indices. However, there was a trend to decrease the autoregulatory property of the hepatic artery, since there was a small increase in slope index and ARI values and decrease in percent change in HAR value. (Appendix A)

Isoproterenol infusion (n=4), ($0.2ug.Kg^{-1}.min^{-1}$ intraportally) significantly dilated the hepatic artery and increased hepatic arterial basal conductance from 0.189 ± 0.019 to 0.247 ± 0.025 $ml.min^{-1}.Kg^{-1}.mmHg^{-1}$ ($P<0.05$). Isoproterenol decreased the hepatic arterial critical closing pressure, however, the decrease was not statistically significant. The isopro- terenol-induced dilation eliminated

the autoregulatory capacity of the hepatic artery. The percent change in HAR was significantly changed from (-16.3 ± 6.3) to (10.1 ± 8.9) ($P<0.05$); ARI increased from 1.07 ± 0.08 to 1.30 ± 0.07; slope index increased from 50.1 ± 9.3 to 80.5 ± 19.2. (Appendix A)

3.3 VALIDATION OF THE SURGICAL PREPARATION

The microsphere study indicated clearly that splanchnic organs still receive some arterial flow after SMA occlusion, i.e. there was still some residual portal flow. This indicates the presence of anastomotic connections between the SMA and other arteries. The data indicated that the non-SMA portal contribution was mainly at the site of the upper third and the cardia of the stomach, and at the lower third of the rectum. In other words, the non-SMA arterial connection sites were mainly at the beginning and the end of the gastrointestinal tract. Complete occlusion of the superior mesenteric artery reduced portal flow to 8.49 ± 1.36% of its control value (portal flow was reduced from 75.28 ± 23.13 to 6.00 ± 1.51 $ml.min^{-1}$; $n=5$) (Appendix C). However, correlation between portal blood flow (calculated from microsphere radioactivity) and SMA flow (correspondingly measured by the flow probe) was extremely poor ($r=0.64$). When portal flow was corrected for by subtracting the residual portal flow (after SMA occlusion), the correlation with SMA flow was highly significant ($P<0.0001$). The correlation coefficient (r) was

0.998. It was concluded, therefore, that changes in SMA flow measured by the use of a flowprobe were representative of changes in portal flow in our preparation. The same microsphere study indicated that the contribution of the hepatic arterial flow to total liver blood flow was 39.63 ± 2.40% (n=5), a value insignificantly different from that presented in Table 1 calculated from flow probe measurements. The microsphere study indicated also that SMA occlusion insignificantly changed cardiac output from 136.62 ± 28.50 to 128.23 ± 25.03 $ml.min^{-1}.Kg^{-1}$ and blood pressure from 111.4 ± 9.7 to 110.6 ± 12.0 mmHg. SMA occlusion reduced the pulse from 226 ± 9 to 218 ± 8 beat/min. This reduction was found to be significant statistically ($P<0.05$) (Appendix C). Since the cardiac output and blood pressure did not change in our preparation during SMA occlusion, the percent of the non-SMA contribution to the portal flow is believed to be the same during the control state.

In an incomplete study, I tried to study HABR in conscious cats using the microsphere technique. Although HABR results were inconclusive (Appendix D), some control data were useful. The spleen contributed 21.79 ± 1.76% (n=6) to total portal blood flow in conscious cats. Clearly removal of the spleen, as with our preparation, would deprive portal flow from a significant contribution of its inflow. The few numbers of animals tested so far would not allow for valid

unpaired statistical comparison between portal (and hepatic arterial) flow during anesthetized and conscious state.

4. DISCUSSION

4.1 GENERAL CONSIDERATIONS

Despite the early observation of the HABR by Burton-Opitz (1911), many subsequent researchers failed to detect such a response. Studies that support the existence of the buffer response were mainly derived from preparations in which the hepatic artery was not long-circuited, whereas, preparations with long-circuited or artificially perfused hepatic arteries showed no or weak HABR (Greenway and Stark, 1971). Therefore, it was essential to have a whole animal preparation with an intact hepatic artery to study the hepatic arterial buffer response and autoregulation. Nevertheless, it should be pointed out that many of our whole animal preparations failed to exhibit the buffer response after the end of the surgical preparation and the stabilization period (30 minutes) that follows. The reason is unclear to us. Postmortem examination of the animals revealed heavy round and/or tape worm infestation in some cases, in others no clear pathological reason could be detected. Regardless of the reasons behind the unsuccessful preparations, we observed that any preparation that failed to show HABR failed to dilate in response to the externally infused adenosine and vice versa. The hepatic artery in such an adenosine non-responding preparation is reactive to other vasoactive compounds such as norepinephrine. On two occasions I infused a low dose of norepinephrine into the hepatic artery, to induce partial constriction, and tested for the recurrence of the adenosine

and the buffer response. Neither one recurred. Around 60% of cats prepared for the quantitation series failed to show HABR and adenosine response. The majority were infested with worms or given antihelmenthic medications recently. Around 50% of cats prepared for the autoregulation series were excluded for the same reason. It seems that the mechanism of the HABR is a sensitive one since re-entry into the abdominal cavity and manipulation of the intestine weakened the buffering capacity in some preparations. Therefore, care should be taken during the surgical preparation to induce as little surgical trauma as possible.

4.2 METHODOLOGY

The use of a whole animal preparation with intact hepatic arterial and superior mesenteric arterial circulation has many advantages and disadvantages. The advantage is to improve the chance of detection of the hepatic arterial buffer response and hepatic arterial autoregulation, and to simulate, as much as possible, the normal physiological situation. However, the preparation we used has many limitations. The inability to raise the superior mesenteric flow (i.e. portal flow) to levels higher than control limits us to testing the hepatic arterial buffer response in one direction, i.e. to decrease portal flow and measure the rise in hepatic arterial flow, whereas, in normal physiological situations portal flow fluctuates above and below control. The dilation of the

superior mesenteric artery by the use of locally infused dilators was not a practical solution to this obstacle since the dilator can reach the liver through portal flow and affect the hepatic artery.

The preparation limited us to test autoregulation within a small range (from control HAP to shoulder). Studies testing autoregulation over a wider range used either the hepatic arterial long-circuit, to be able to increase the perfusion pressure, or used the isolated perfused liver preparation (Greenway and Stark, 1971). The use of the arterial long-circuit can render the hepatic artery less reactive (Greenway et al., 1967). To raise hepatic arterial pressure head without the use of a hepatic arterial long-circuit, occlusion of the aorta below the kidneys was applied. This procedure was tried before by Stokland et al., (1981) and was shown to produce elevation in arterial pressure with no effect on cardiac output, stroke volume or myocardial shortening. However, Stokland's group made the occlusion of the aorta above the kidneys. We occluded the aorta below the kidneys to avoid the possibility of the stimulation of the renin-angiotensin system that might affect the validity of our results. The rise in pressure in this situation is due to the increase in peripheral resistance, a situation similar to some physiological hypertensive states. Our data showed that aortic occlusion did not affect the basal parameters of the hepatic artery and did not affect the shape of the pressure-

flow curve. However, the aortic occlusion showed a trend to shift the curve up and to the left slightly. This observation is similar to that made by Torrance (1961) with pharmacological denervation of the liver. Nevertheless, the mean rise in systemic blood pressure was 13-14mmHg, a finding similar to that observed with occlusion of the aorta above the kidneys (Stokland et al., 1981).

For the superior mesenteric flow to be synonymous with portal flow, splenectomy was a prerequisite. The splenic artery is a branch of the celiac artery (Figure 6) and there is no anastomotic connections between the splenic circulation and the superior mesenteric artery such as the case with the superior and inferior mesenteric artery. Therefore, ligation of the splenic artery alone would end with ischemia of the whole spleen. Moreover, it is difficult to approach the area of the celiac artery, the superior mesenteric artery and the splenic veins to apply flow probes and vascular occluders, and to cannulate some splenic veins, while the spleen is still in place. The disadvantage of splenectomy, on the other hand, is that it deprives portal flow from a significant amount of portal input. Our data indicated that the spleen contributes 22% to total portal blood flow in conscious cats (Appendix D). Clearly this partial reduction in portal blood flow might stimulate some increase in hepatic arterial flow (HABR). This is evident from control data presented in Table 1. The hepatic arterial contribution to total liver blood flow was

around 40% in our preparation compared to 25% in others (Greenway and Stark, 1971; Mathie and Blumgart, 1983). Therefore, data obtained from such a preparation may underestimate the control value of the HABR.

Measurement of portal blood flow by direct application of a flow probe on the portal vein has been applied by many research workers in different kinds of animal species. However, the attempt to try such a procedure in our laboratory on cats failed to give consistently reliable measurements. Application of the flow probe on an artery gives much more stable and reliable measurements compared to that of a vein. It seems that the collapsible nature of the veins does not allow proper intimate contact between the portal vein wall and electrodes of the flow probe. Such intimate contact is essential for the conduction of the electromagnetic signal from the flow probe to the flowmeter. In addition, portal venous flow is difficult to accurately calibrate. To avoid this obstacle, we applied the flow probe on the superior mesenteric artery with the inferior mesenteric artery, gastroduodenal artery and gastric artery being ligated. Therefore, portal flow in our preparation is derived almost entirely from the superior mesenteric artery. Nevertheless, this methodology should be validated to prove that arterial flows, other than that from the superior mesenteric artery, have no significant contribution to portal blood flow. To do so, in a pilot study, two radioactively labelled (^{57}Co and

^{113}Sn) microspheres (16U in diamter) were injected at the ascending arch of the aorta. One batch was administered before the SMA occlusion to serve as a control, the second was administered after the occlusion. Our data indicated clearly that occlusion of the SMA in our preparation did not completely eliminate the portal flow (Appendix C). Nevertheless, the non-SMA contribution to portal flow did not cause any significant error in calculating SMA flow changes. The lack of existence of any ischemic regions in the splanchnic organs, in our preparation, suggests strongly that the anastomotic connections between the SMA and other splanchnic arterial beds were efficient. Ligation of the inferior mesenteric, gastric and gastroduodenal arteries were fully compensated for through the anastomotic connections with the SMA. However, some non-SMA flow was still reaching the gastrointestinal tract mainly at its two ends. It seems that some fine arteries, directly originated from the abdominal aorta, are responsible for this non-SMA contribution. It is interesting to note that these very sites are the sites of anastomosis between portal vein and systemic venous circulation. These anastomosis are known to shunt portal blood to the systemic venous circulation during some portal hypertension cases.

The microsphere study indicated as well that occlusion of the SMA has no significant effect on hemodynamics. Cardiac output and systemic blood pressure showed insignificant

changes in response to the SMA occlusion. The study supported the observation that the proportion of hepatic arterial flow to total liver blood flow is higher in our preparation (40%) compared to the 25% proportion obtained from non-splenectomized preparations (Mathie and Blumgart, 1983).

Despite some limitations, our data and the discussion presented above support the conclusion that the preparation and methodology used in this research work were valid and capable of testing the HABR and pressure-flow autoregulation in the hepatic artery, as well as their mechanism of action.

4.3 THE QUANTITATION OF THE HEPATIC ARTERIAL BUFFER RESPONSE

Despite the frequent demonstration of the HABR by many investigators, no real attempts have been made to quantitate the buffering efficiency of the hepatic artery in a systematic way. This lack of information might be attributed to the difficulty in correlating between the hepatic arterial flow and portal venous flow in the presence of many variables. Within the same experimental procedure, the control hepatic arterial flow and conductance can change in response to alterations in cardiac output, systemic blood pressure, circulating blood volume or portal flow. Therefore, quantitation of the HABR should be conducted in a model that can maintain these parameters as steady and stable as possible. It is evident from our data that the hepatic arterial buffering efficiency, in response to wide range

changes in portal flow, was not constant even when the hepatic arterial perfusion pressure was held steady. The highest buffering efficiency of 24.24% was reached at 60% decrease in portal flow, i.e. 24% of the reduction in portal flow was compensated for by the increase in hepatic arterial flow (Figure 9). Further reductions in portal flow were less compensated for by the hepatic artery. However, when the hepatic arterial perfusion pressure was allowed to rise during the induction of the HABR (i.e. uncontrolled HAP), the buffering efficiency of the HA was constant with an average compensatory capacity of 30.30% (Figure 11). Some previously available information had indicated, without quantitation, a non-linear relationship between changes in hepatic arterial flow and portal flow. Hanson and Johnson (1966) found that hepatic arterial flow tended to reach a plateau value at very low portal venous flows. Recalculating their mean data showed that a peak HABR of 8% was reached at 66% decrease in portal flow, when portal blood flow was further decreased the buffering efficiency decreased as well. Considering that HAP decreased during their procedure and the HA and portal vein were long-circuited, the authors data were surprisingly in close agreement with our findings. In contrast with Hanson and Johnson's (1966) findings, Gelman and Ernst (1977) observed that the increase in hepatic arterial flow became more and more pronounced as portal venous flow was reduced, i.e. the more the reduction in portal flow the more efficient

the HA became to buffer portal flow changes. However, the hepatic hemodynamic changes recorded from this study were induced by the direct infusion of acids and sodium bicarbonate into the portal vein. This infusion altered significantly the composition of portal blood and affected the systemic arterial and venous blood pH. The same study showed that a decrease in portal blood pH led to an increase in hepatic arterial flow when portal flow was held steady, whereas lowering of arterial blood pH due to the intraportal infusion of hydrochloric or lactic acid caused significant reduction in portal blood flow and an increase in hepatic arterial flow. An induced increase in portal blood pH led to an opposite but weaker change in hepatic arterial flow. Although the reason behind the pH induced reduction in portal blood flow was unclear, nevertheless, it seems possible to conclude that the increase in the buffering efficiency at lower portal flows might be attributed to the additional dilation of the hepatic artery in response to the H^+ ion delivery through portal blood. Despite the employment of an in vivo artificial perfusion of the liver by the above two groups of authors, it is likely that the more physiological preparation of Hanson and Johnson may have yielded a more representative set of results.

The best attempt to quantitate the HABR, so far, was the study of Mathie and Blumgart (1983). This group of authors evaluated the buffering capacity of the hepatic artery in dogs that were anesthetized and had undergone the acute stress of

establishment of a side-to-side mesocaval anastamosis. This preparation allowed the controlled reduction of portal flow without inducing any reduction in venous return, hence, systemic blood pressure was held steady. Although the hepatic arterial response to portal flow changes was tested over a wide range of acute altered portal venous flow, the HABR was not related to gradual or stepwise reductions in portal blood flow. Therefore, data points were scattered between the minimum and maximum portal flow level reached. Data points were found to fit a linear relationship with a line of best fit equation: $Y = 0.24x - 0.32$; where Y and x signify hepatic arterial and portal flow changes respectively. Hence, the pooled buffering capacity was 24%. The authors recalculated their data to quantitate the buffering efficiency from each individual experiment. The mean buffering efficiency value calculated from their scattered HABR data was $22.7 \pm 2.0\%$, a figure very close to 24.24% derived from our data at 60% decrease in portal flow. However, the authors neither indicated whether individual data points of buffering efficiency were statistically insignificantly different from each other, nor revealed whether the linear relationship between portal flow alterations and hepatic arterial flow response was statistically significant. Nevertheless, the results derived from the Mathie and Blumgart (1983) preparation are in close agreement with our pressure-uncontrolled HABR data where buffering efficiency was found to

be steady with a mean value of 30.30%, despite the difference in species, preparations, and methods of induction of the HABR.

In this study the buffer capacity of the hepatic artery was quantitated in the form of absolute and percent change in hepatic arterial conductance as well. The hepatic arterial conductance (HAC) reached a plateau value at 60% decrease in SMAF (portal flow) (Figure 13), whereas the percent change in HAC revealed a continuous increase in response to the progressive decrease in portal blood flow (Figure 12), to reach a peak value of 28.68% at complete occlusion of the superior mesenteric artery. Our finding is similar to that of Hanson and Johnson (1966), who obtained a curvilinear relationship between portal flow and hepatic arterial resistance, but different from that calculated by Richardson and Withrington (1978) who observed a linear association between portal venous pressure (and flow) and hepatic arterial resistance. However, it should be pointed out that the latter authors employed pump operated hepatic arterial and portal venous long-circuits in their preparation. Recalculation of their mean data to compare them with ours revealed significant differences. The basal hepatic arterial resistance was close to 16 compared to 5 $mmHg.ml^{-1}.min.Kg$ obtained from our data. Their critical closing pressure was extremely high (40mmHg) compared to ours and 100% occlusion of portal venous flow caused about 15% increase in HAC and 11% HABR capacity, values

close to half of that obtained from our preparation, indicating weak buffering efficiency. These findings are in agreement with the observation made before (Greenway and Stark, 1971) that long-circuited preparations demonstrate weak or absent buffering efficiency in the hepatic artery. However, there would seem to be general agreement that increases in hepatic arterial conductance are intimately related to the increases in hepatic arterial flow observed when portal venous flow is reduced. The presence of some degree of pressure-induced hepatic arterial autoregulation during pressure-uncontrolled HABR may support the observation of the mutual existence of hepatic arterial autoregulation and HABR.

The hepatic buffering efficiency range of 24-30% obtained from our research work does not differ from the average buffer capacity of 25% seen in several series conducted in our laboratory. A compensation for one-quarter of a reduction in portal flow does not sound physiologically significant for maintaining total liver blood flow rates. Such criticism is quite valid, however, several facts suggest that the buffer capacity of the hepatic artery is underestimated by the methods used to study the response. The exposure of animals to the anesthesia and the acute surgical stress result in mesenteric vasoconstriction (McNeill and Pang, 1982), and subsequently lower portal blood flow. Further reduction in portal flow would be added due to splenectomy. Therefore,

portal blood flow would be unusually low. This would partly trigger the buffer response before we test for its presence. It has been pointed out already that the contribution of the hepatic artery to total liver blood flow in our preparation (40%) was higher than that (25%) obtained from other studies. This finding is in support with the existence of partially stimulated HABR in our preparation. Thus, while the described methods were suitable for producing a reproducible response for mechanistic studies, the quantitative value of the buffer is not physiologically relevant.

The hepatic arterial buffer response can be observed and quantitated in many studies, although the HABR was not being studied per se. The methods used in these studies were widely variable and ranged from anesthetized acute preparations to conscious chronic ones. Hemodilutions (Lautt, 1977b) and metabolic inhibitors and stimulators (Lautt, 1980) produced alterations in portal flow, with the hepatic arterial flow compensating to hold total blood flow steady. Hughes et al. (1980) produced lactic acidosis in dogs and observed an increase in portal blood flow and a dramatic decrease in hepatic arterial flow, resulting in no change in total hepatic blood flow, i.e. 100% HABR capacity. This complete compensation of the hepatic artery took place despite the fact that oxygen delivery to liver cells and hepatic venous oxygen content declined. Lagerkranser et al. (1984) tested the effectiveness of adenosine for inducing controlled

hypotension. Despite the direct vasodilatory effect of adenosine on the hepatic artery, intravenous or intra-arterial infusion of adenosine caused 64% decrease in preportal vascular resistance, while hepatic arterial resistance increased by 49% (i.e. vasoconstriction). The increase in portal blood flow was completely compensated for by the decrease in hepatic arterial flow resulting in no change in total hepatic blood flow as well. Similar conclusions can be derived from several conscious preparations. In two cirrhotic rat models (Fernandez-Munoz et al., 1985; Lebrec and Blanchet, 1985), the hepatic arterial blood flow was altered to offset changes in portal blood flow induced by the cirrhotic processes. In one model (Fernandez-Munoz et al., 1985), which exhibited no significant porta-caval shunts, portal blood flow was significantly elevated and the hepatic arterial flow was reduced sufficiently to hold total hepatic blood flow at similar levels in the control and cirrhotic animals. All other arterial flow (muscle, testes, kidneys and splanchnic organs) were elevated and conductances were increased. The vasoconstriction of the hepatic artery was unique since all other arteries showed vasodilation, implying that the buffer overcame some general systemic dilator influence and yet produced an impressive compensation. The other model (Lebrec and Blanchet, 1985) produced biliary cirrhosis and portal hypertension. Portal flow was reduced, due to reduced splanchnic blood flow, and the hepatic arterial flow was

elevated. The increase in the hepatic arterial flow fully compensated for the reduction in portal flow and held total liver blood flow at a level similar to that of control, i.e. it exhibited 100% buffer capacity. The majority of the data presented above give the impression that the buffer capacity may be considerably more effective in compensating for portal flow elevations than for reductions in portal flow, and that the buffer capacity may be higher in conscious preparations than in anesthetized ones. Unfortunately, the limitations and the design of our preparation did not allow us to test such a speculation.

Data derived from several hemorrhagic studies suggest strongly the presence of an effective HABR, however it is difficult to quantitate the response. In most of these studies the cardiac output decreased during hemorrhage and total hepatic blood flow significantly decreased, despite the evident dilation of the hepatic artery (Greenway and Stark, 1971). After hemorrhage, portal flow decreased due to arteriolar vasoconstriction in the spleen and intestine, however, total hepatic flow decreased approximately in proportion to the decrease in cardiac output. The decrease in portal flow was proportionately greater than the decrease in total hepatic blood flow (Greenway and Stark, 1971). The hepatic arterial flow was either unchanged (Mundschau et al., 1966) or increased (Slater et al., 1973) in spite of the fall in arterial pressure head. Thus, after hemorrhage the hepatic

arterial flow formed a higher proportion of the total hepatic flow. Replacement of the hepatic arterial and portal venous flows by their corresponding percentage of cardiac output showed that during hemorrhage the decrease in portal flow percentage of cardiac output was compensated for by the increase in hepatic arterial percentage of cardiac output. Hence, during hemorrhage the hepatic artery holds the total hepatic flow percentage of the cardiac output steady (Slater et al., 1973; Kaihara et al., 1969). This peculiar role of the hepatic artery during the reversible hemorrhagic shock state raises the speculation that, when the buffer capacity fails to maintain a stable total hepatic flow in the face of reduced cardiac output and blood pressure, it maintains a constant proportion of the cardiac output for the hepatic vascular bed.

4.4 ADENOSINE AND ADENOSINE RECEPTOR BLOCKERS

4.4.1 Adenosine

Adenosine is one of the purine compounds whose profound hypotensive, sedative, antispasmodic, and vasodilatory actions were first recognized over five decades ago (Drury and Szent-Gyorgi, 1929). Over the years the number of biological roles suggested for adenosine and for precursor adenine nucleotides have increased tremendously. Extracellular adenosine receptors and adenine nucleotide receptors have been identified (Bruns et al., 1980; Schrader et al., 1977). In

many cells the adenosine receptors appear linked to adenylate cyclase, while nucleotide receptors (such as ATP receptors) probably control ion fluxes (Fox and Kelley, 1978). Adenosine may represent a general regulatory substance, since many cell types seem capable of synthesizing it. Unlike various endocrine hormones, there is no evidence for storage and release of adenosine from nerves or other cells. The presence of purinergic nerves has been proposed in peripheral systems (Burnstock, 1978), but ATP rather than adenosine has been considered the neurotransmitter in such nerves.

Adenosine has different physiological roles in different organs. In the cardiovascular system, intravenous (or intra-arterial) infusion of adenosine leads to arterial vasodilation and hypotension (Lagerkranser et al., 1984), and also leads to cardiac depression (i.e. negative chronotropic and inotropic effects) (Prasad et al., 1980). Unlike other vascular beds, renal arterioles constrict in response to adenosine infusion (Hedqvist et al., 1978). The antispasmodic and vasodilatory actions appear linked to smooth muscle depressant effects of adenosine. Such depressant effects are perhaps linked to activation of muscle adenylate cyclase (McKenzie et al., 1977). Adenosine has inhibitory effects on neurotransmission and on spontaneous activity of central neurons. The mechanisms are unknown but certainly involve calcium and may involve presynaptic inhibition of adenylate cyclase (Hedqvist et al., 1978; Phillis et al., 1979; Stone, 1981a). The

reversal of the inhibitory effects of adenosine on cholinergic and noradrenergic transmitter release by calcium ions has led to adenosine and its analogues being referred to as calcium antagonists (Silinsky, 1981; Stone, 1981b). Less attention has been given to the antilipolytic and antithrombotic action of adenosine compared to the cardiovascular and neural function effects of adenosine.

The information available on the physiological roles of adenosine in the hepatic circulation is scarce. Most of the studies were directed to understand the roles of adenosine or its analogues on hepatocyte biological processes. However, it has been pointed out in the introduction that hepatic blood flow is not linked to its metabolic processes, and that the anatomical arrangment of the acinus does not allow any biological end products released from the hepatocytes to get access to resistance sites upstream. Externally administered adenosine causes vasodilation of the hepatic artery (Greenway and Stark, 1971). In contrast with the reduced oxygen uptake by the myocardial and preportal tissues, the liver responded to adenosine infusion by an increased oxygen uptake. In other words, the increased hepatic oxygen uptake shows that the liver responds in a different manner from other tissues exposed to exogenous adenosine (Lagerkranser et al., 1984). This may be explained by an enhancement of the oxygen consuming process in the hepatocytes, such as increased gluconeogenesis (Haeckel, 1977), stimulation of hepatic

adenylate cyclase (Schutz et al., 1982) and incorporation of adenosine and its metabolites into intracellular ATP.

The liver is a major supplier of adenosine to the systemic circulation. Adenosine levels in hepatic venous blood are tenfold portal and arterial levels (Pritchard et al., 1975). The liver effectively removes 80% of hypoxanthine, xanthine and urate (i.e. oxypurines) in a single passage of blood through the liver and converts them to utilizable purine (adenosine) (Pritchard et al., 1975). It is believed that this exported adenine derivative is utilized by cells and tissues (such as erythrocytes, leukocytes, gastrointestinal mucosa and bone marrow) which are totally incapable, or at least markedly defective, in de novo purine synthesis despite having a high rate of purine turnover (Pritchard et al., 1975). However, it is unclear yet which cell type in the liver is responsible for the clearing of hypoxanthine from afferent blood and the simultaneous addition of adenosine to efferent blood. The hepatic arterial buffer response cannot be explained according to this physiological adenosine production from the liver. First, the adenosine release is downstream, while the vascular resistance sites are upstream. Second, the efficiency of hypoxanthine conversion to adenosine was maintained in isolated perfused liver preparation, a situation where the buffering capacity is known to be markedly attenuated.

Two mechanisms to explain adenosine release from cells have been proposed. One possible mechanism is that adenosine is made at the surface of cells by 5'-nucleotidase bound to the plasma membrane (i.e. an ecto enzyme) (Frick and Lowenstein, 1978; Berne, 1980). This enzyme is known to be present in hepatocyte membrane (Drummond and Yamamoto, 1971). The other possibility is that adenosine is made inside the cell and leaves via passive and facilitated diffusion (Schutz et al., 1981). The metabolic pathways involved in formation and inactivation of adenosine are still poorly resolved questions. Two metabolic pathways have been suggested (Figure 20). Certainly, the most obvious pathway for formation of adenosine involves ATP → ADP → AMP as precursors and might be called the "ATP Pathway". Formation of adenosine via this ATP pathway appears to increase considerably in cells under conditions of high energy demands (i.e. there is energy deficit) (Arch and Newsholme, 1978). Under such conditions, 5'-AMP levels increase intracellularly as levels of ATP decrease due to excessive energy demands. Endo-5'-nucleotidase, thought to be freed from inhibition by ATP, hydrolyzes 5'-AMP to adenosine. Adenosine can then cross biological membranes either actively or passively to reach extracellular receptors. The ATP pathways can also occur extracellularly , and this may, in some tissues, be a very important route to adenosine. For example, ATP coreleased with a neurotransmitter, such as norepinephrine or

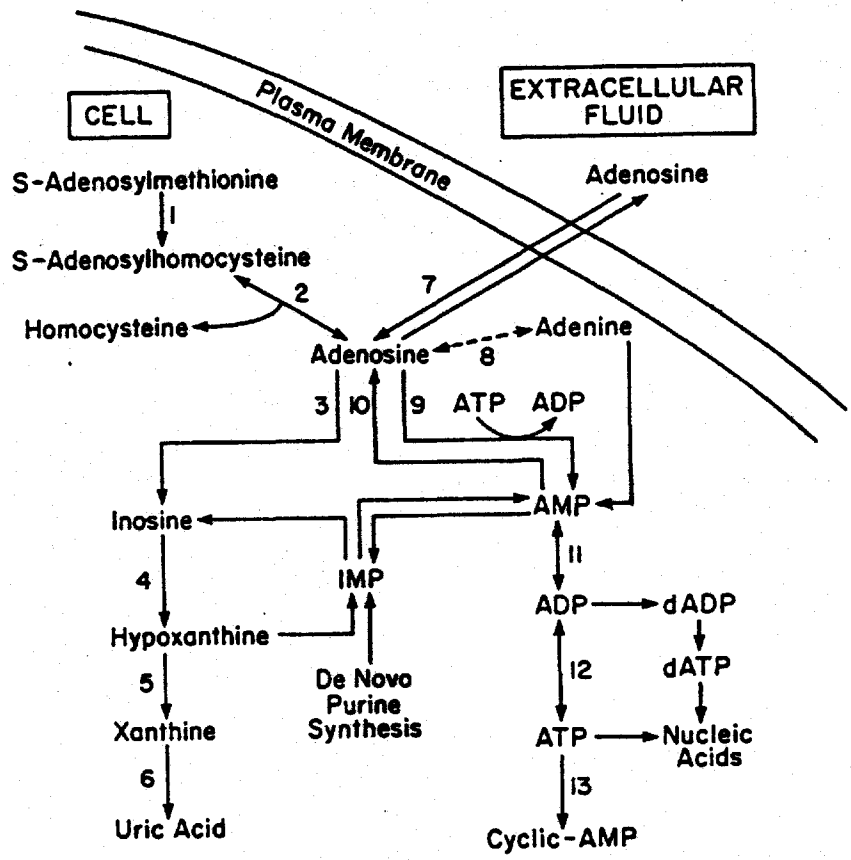

Fig. 20 Metabolism of adenosine. This diagram illustrates the pathways by which adenosine is formed and degraded within cells. Adenosine may also be transported into cells or released from cells: 1, S-adenosylmethionine methyltransferases; 2, S-adenosylhomocysteine hydrolase; 3, adenosine deaminase; 4, purine nucleoside phosphorylase; 5 & 6, xanthine oxidase; 7, transport mechanisms; 8, adenosine phosphorylase (not established); 9, adenosine kinase; 10, 5'-nucleotidase and non-specific phosphatase; 11, adenylate kinase; 12, nucleoside diphosphokinase; 13, adenylate cyclase (Fox and Kelley, 1978).

acetylcholine, can either act at ATP receptors or can be hydrolyzed to adenosine by ecto-ATPases and ecto-5'nucleotidase. Similarly, ATP might be released as a transmitter itself to act on receptors then be hydrolyzed to adenosine. Inhibition of the extracellular (membrane-bound) ATP pathway can be achieved through the blockade of ecto-ATPases and ecto-5'-nucleotidase, whereas attenuation of adenosine release from the intracellular ATP pathway can be achieved by blocking the adenosine transport system by dipyridamole and nitrobenzylthioinosine (NBTI) (Belloni et al., 1985). Since dipyridamole and NBTI inhibit adenosine uptake (i.e. influx) as well, therefore, they can be considered as bidirectional adenosine transport blockers.

Other than the physiological production of adenosine by the liver, isolated hepatocytes release adenosine during hypoxic conditions. This adenosine release from hypoxic hepatocytes can be attenuated by inhibitors of facilitated adenosine transport, but not by inhibition of ecto-5'-nucleotidase (such as, ,B-methylene adenosine diphosphate) (Belloni et al., 1985). Therefore, the intracellular ATP pathway is believed to be responsible for the hypoxic release of adenosine by hepatocytes. These findings support the speculation that hepatocytes produce and release (i.e. export) adenosine all the time, and that adenosine release is enhanced during periods of hypoxia. This export enhancement might be important to the dependent peripheral tissue for the

replenishment of their cellular mucleotide levels upon regaining a normoxic state. The adenosine hypoxic response cannot explain the mechanism of the HABR, since the HABR is not coupled to the oxygen demand of hepatocytes as discussed earlier in the introduction.

A second possible cellular pathway to adenosine formation which does not involve ATP pathway (i.e. not coupled to oxygen demand), was proposed, namely, the "methylation pathway". Biological methylation in intact cells leads to the generation of S-adenosylhomocysteine, which is converted by the enzyme S-adenosylhomocysteinase to adenosine and homocysteine (Figure 20). It should be noted that this reaction is readily reversible. The methylation pathway, unlike the ATP pathway, can serve not only to generate adenosine but can, in the presence of homocysteine, serve to maintain very low levels of the nucleoside within the cell. Other enzymes whose function may be to maintain low levels of intracellular adenosine are adenosine deaminase (converts adenosine to inosine) and adenosine kinase (converts adenosine to 5'-AMP).

Under normal conditions adenosine levels, both intra and extracellularly, seem to be low. A variety of routes for inactivation of adenosine are present in cells. These include enzymes, such as adenosine deaminase, adenosine kinase, and S-adenosylhomocysteinase, and uptake processes. These pathways maintain low levels of endogenous adenosine (1-2mM or less under most physiological conditions), extracellular adenosine

levels probably also never rise under normal conditions above 1-2mM (Daly, 1982). Although cells produce adenosine to serve certain regulatory roles, they inactivate adenosine quickly in order to attenuate its cytotoxic property. It has been suggested that irreversible interactions with S-adenosylhomocysteine may represent the molecular loci for the cytotoxicity of adenosine and adenosine analogues (Hershfield and Kredich, 1978). Adenosine and adenosine analogues can irreversibly inhibit the enzyme S-adenosylhomocysteinase as well (Hershfield and Kredich, 1978). The resultant accumulation of analogues of S-adenosylhomocysteine causes an inhibition of methylation pathways within the cells. The relative importance of the catabolic and anabolic pathways for adenosine in hepatic cells has not been well defined and further investigation is undoubtedly required.

Adenosine deaminase converts adenosine to an inactive metabolite, inosine. Inosine is further metabolized by phosphorylases to hypoxanthine. Potent inhibitors for adenosine deaminase are available, namely, deoxycoformycin which is an irreversible inhibitor and erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) which is a reversible inhibitor (Plunkett et al., 1979; Skolnick et al., 1978). These deaminase blockers are specific in their action and seem better than adenosine uptake (or transport) inhibitors, such as dipyridamole, in potentiating adenosine responses. Combination of adenosine uptake and deaminase inhibitor causes

an additive, and not multiplying, effect (Henderson et al., 1977). It should be pointed out that many adenosine analogues, such as 2-choloradenosine, are not substrates for adenosine deaminase.

The other enzyme involved in the inactivation of adenosine, namely, adenosine kinase, converts adenosine to 5'-AMP, which then enters into the metabolic pool of adenine nucleotides. However, inhibitors of this enzyme have not been widely used (Daly, 1982).

In addition to the enzymatic inactivation, adenosine is subject to a high affinity uptake system which removes it from the extraceullar compartment and, hence, from getting access to extracellular adenosine receptors. Such an uptake system may actually be tightly coupled to sites of extracellular generation of adenosine and to the enzymes involved in intracellular inactivation of adenosine. Inhibitors of the adenosine-uptake system include dilazep, dipyridamole, hexobendine and papaverine. Some Ca^{++} channel blockers (such as lidoflazine, flunarizine and nifidipine) are potent adenosine transport inhibitors as well (Daly, 1982). Such compounds potentiate adenosine responses, however, they should be used cautiously, since most have other biological activities, including activity as phosphodiesterase inhibitors (Fredholm, 1978).

Our data indicated clearly that the HABR and the hepatic arterial autoregulation are adenosine mediated. The logical

question at this stage is how to explain the washout theory according to the discussion presented above regarding adenosine physiology and mechanisms of formation. The existence of two pathways to generate adenosine and the fact that the HABR is not coupled to the oxygen demand or supply, allows us to speculate that there are two independent pools for adenosine release within the liver. One pool is at the level of sinusoids where hepatocytes (and probably some other cells such as Kupffer cells) clear afferent blood of hypoxanthine, xanthine and urate (released from other peripheral tissue cells) and continuously release adenosine in hepatic venous blood (efferent blood) and that this release of adenosine increases in cases of hypoxia or energy deficit where more adenine nucleotide derivatives are formed within the cell. The mechanism of formation of this adenosine pool might be through the ATP-pathway since it can be linked to oxygen and energy demand. The other pool of adenosine is at the space of Mall close to the hepatic arteriole resistance sites. Adenosine is released continuously, and at a constant rate regardless of the oxygen demand, in this pool from specialized cells within the space of Mall. This locally produced adenosine either gets access to act on adenosine receptors on resistance sites of the hepatic arterioles, or is washed away from the vicinity by the portal flow or hepatic arterial flow (or even lymphatic flow) (Figure 5). Therefore, the concentration of adenosine in this pool is regulated by

hepatic blood flow rather than by oxygen demand. The mechanism of generation of adenosine from these specialized cells might be according to the methylation pathway. Clearly much research remains to be done to determine the roles of adenosine in hepatic hemodynamics. Very little is known about the physiology of the space of Mall and its limiting plate. The exploration of this area may be quite significant for the proper understanding of blood flow regulation within hepatic sinusoids.

4.4.2 Adenosine Receptors

The extracellular adenosine receptors are referred to in some literature as P_1 receptors to differentiate them from the adenine nucleotide (P_2) receptors (i.e. ATP receptors). It has now been recognized that there is not one but at least two classes of adenosine receptors. One of these has a high affinity for adenosine and, at least in some cells, couples to adenylate cyclase in an inhibitory manner. These have been termed the A_1 receptors by Van Calker et al., (1979), and the R_i receptors by Londos et al., (1980). The second class of receptors has a lower affinity for adenosine and, in many cell types, couples to adenylate cyclase in a stimulatory manner. These have been termed the A_2 receptors or R_s receptors. In this thesis, the two classes of adenosine receptors are referred to as A_1 and A_2 receptors. Most, but perhaps not all, adenosine receptors appear linked to adenylate cyclase. At present very few actions of adenosine can be stated with

assurance not to involve cyclic AMP system. One such action is the stimulation of glucose oxidation in adipocytes by adenosine and analogues (Souness and Chagoya de Sanchez, 1981). This action does not appear to involve cyclic AMP systems. The stimulatory A_2 receptor is more ubiquitous in occurrence compared to the inhibitory A_1 receptor (Bruns, 1980). A_1 receptor is present in adipocytes and heart and brain cells (Daly, 1982). The adenosine receptors in the liver seem to be of the A_2 class (Londos et al., 1980).

The best known antagonists (blockers) of adenosine receptors are alkylxanthines, such as theophylline and caffeine (Smellie, Davis et al., 1979). Unfortunately, no selective xanthine antagonists for either A_1 or A_2 adenosine receptors have been developed yet. An 8-phenyl substituent to theophylline confers very high affinity at both A_1 and A_2 receptors. The following list is the rank order of potencies of some xanthines versus both A_1 and A_2 adenosine receptor mediated effects on adenylate cyclase. 8-Phenyltheophylline (8-PT) > 1,3-dibutylxanthine > 3-isobutyl-1-methylxanthine (MIX) > theophylline > caffeine > theobromine (Daly, 1982). The adenosine receptor blocker 8-PT is twenty-fold more potent than theophylline. The inhibition constant for 8-PT is about 0.2uM for both A_1 and A_2 receptor, whereas MIX inhibition constant is about 2uM for A_1 receptor and 5uM for A_2 receptor (Daly, 1982). Therefore, 8-PT is much more potent than MIX. Many of the xanthines have other biological activities, in

particular, inhibitory effect on phosphodiesterases (Smellie, Davis et al., 1979). The inhibition of this enzyme prevents intracellular degradation of cyclic AMP and results in an increase in intracellular concentration of cyclic AMP. This phosphodiesterase inhibition complicates the use of xanthines as selective adenosine receptor blockers. Our data are in agreement with the above information. 8-PT was a powerful adenosine receptor blocker compared to MIX, it can be given in bolus doses while MIX administration requires its continuous infusion. Theoretically an adenosine receptor blocker should constrict the hepatic artery. 8-PT led to some vasoconstriction (Table 1; the unraised pressure series), whereas MIX administration led to significant dilation of the hepatic artery, reduced the total peripheral resistance and decreased the systemic blood pressure. This vasodilation of the hepatic artery in response to MIX infusion, might be explained according to its powerful phosphodiesterase inhibiting property. The resultant accumulation of cyclic AMP within the smooth muscle cells of the hepatic arterioles led to a dilator effect that counters the expected constrictor responses resulting from adenosine receptor blockade. Certain xanthines, close in their chemical composition to MIX, such as 1-isoamyl-3-isobutylxanthine, are known to be much more potent as phosphodiesterase inhibitors than as adenosine antagonists (Smellie, Daly et al., 1979). Recently, 8-(p-sulfophenyl) theophylline (8-SPT) has been introduced as a polar adenosine

antagonist which should penetrate cells only to a limited extent (Petrack et al., 1981). Unlike 8-PT, this polar derivative is not particularly potent, being comparable in potency to theophylline itself (Daly, 1982). Our data agree with this finding. High dosage was required to reach the blocking range. In some preparations the blocking range could not be reached since we reached the saturation point of the 8-SPT solution and higher concentrations could not be achieved even with changing the pH and temperature of the solution. Therefore, 8-SPT was substituted with 8-PT in those preparations. We do not recommend the use of 8-SPT in whole animal preparations basically for two reasons. First, it is much less potent than 8-PT and second, it is quite expensive. Continuous infusion of 8-SPT (similar to MIX) is uneconomical and futile. Despite its adenosine receptor blocking property, 8-SPT did not induce vasoconstriction in the hepatic artery similar to 8-PT. However, our data cannot exclude the presence of some inhibitory effects on phosphodiesterases or any other biological activites. So far 8-PT seems to be the best xanthine antagonist available to study adenosine receptor blockade. It should be pointed out that 8-PT is competitive and specific in its action on adenosine receptors. It does not alter responses to other vasoactive substances such as norepinephrine and isoproterenol (Lautt and Legare, 1985).

4.5 THE MECHANISM OF HABR AND AUTOREGULATION

The unified hypothesis tested is that the reduction in hepatic arterial resistance that occurs in response to reduced portal venous or hepatic arterial flow is dependent upon the reduced portal or arterial flow washing away less adenosine; the adenosine accumulation leads to vasodilation. Figure 5 explains the putative mechanism of the HABR and autoregulation. Our results indicated clearly that HABR and autoregulation were adenosine mediated. Adenosine receptor blockers (MIX, 8-PT and 8-SPT) inhibited both the dilatory response to the externally administered adenosine and the buffering capacity (Figures 14,15,17). Restoration of the dilator response to infused adenosine was accompanied always by restoration of the buffer response. The inhibition of the HABR was evident regardless of the criterion of quantitation. Figure 14 expresses the inhibition of the HABR as a decrease in percent change in HAC, whereas Figures 15 and 17 express the inhibition as a reduction in the buffering efficiency. It can be argued, however, that MIX inhibition of the buffer capacity can be attributed to the MIX-induced maximal dilation of the hepatic artery. No adenosine dose-response plots have been conducted in this study to show the maximal dilation of the hepatic artery, and thereafter to conclude whether the hepatic artery was maximally dilated during MIX infusion or not. Nevertheless, such a test has been conducted elsewhere (Lautt et al., 1985). MIX did not produce a maximally dilated

hepatic artery during its infusion in that study. Despite the above mentioned side effects of MIX, the similarity between its inhibitory effects on adenosine infusion and HABR, and other adenosine receptor inhibitors (8-PT and 8-SPT), suggests that the MIX inhibition was related to its adenosine receptor blockade property per se.

Since the quantitation and HABR mechanism series have shown that adenosine mediated the HABR, and to test whether the dilator washout theory can explain autoregulation of the hepatic artery as well, the autoregulation series was designed. Our basic protocol was to obtain arterial vasodilation to intraportal infusion of a test dose of adenosine (0.4 mg/Kg per min), then to obtain and quantitate a pressure-flow curve and a HABR and then to determine the effect of selective, competitive adenosine antagonists on these responses. Figure 15 shows a dose-related effect of two bolus doses of 8-PT on the pressure-flow curve and on the two indices of adenosine receptor blockade. The HABR and response to infused adenosine show dose-related antagonism consistent with previous results (Lautt and Legare, 1985) and the autoregulatory curves show graded blockade with the high dose of 8-PT producing a nearly linear curve indicative of lack of autoregulation. A similar effect on autoregulation, the HABR and the response to adenosine infusion are seen in pooled results from six cats in which blocking agents had inhibited the response to infused adenosine and the HABR. It is clear

from Figures 15, 17, and 19 and Table 2 that, regardless of the means of data expression, autoregulation was blocked by these methylxanthenes. The response of the hepatic artery to isoproterenol and norepinephrine are not antagonized by 8-PT, showing the selective nature of the antagonism and indicating that the artery can still dilate to other stimuli (Lautt and Legare, 1985).

For adenosine to serve as the intrinsic dilator of the washout hypothesis, four criteria should be met. First, it should be a powerful dilator of the hepatic artery. Second, portal blood must have access to the hepatic arterial resistance vessels, i.e. intraportal infusion of adenosine should dilate the hepatic artery. Third and fourth, inhibition and potentiation of exogenous adenosine responses should also inhibit and potentiate the buffer response and autoregulation respectively. Our results showed clearly that the first three criteria have been met (Figures 14,15,17; Table 2). In a previous study (Lautt et al., 1985), dipyridamole (adenosine transport inhibitor) increased the buffering efficiency from a control value of 23% to 34.2%. In an unsuccessful series, I administered dipyridamole intra-arterially (the hepatic artery) to test for autoregulation. Instead of potentiating the HABR and autoregulation, dipyridamole actually inhibited both responses as well as the response to infused adenosine. Reduction of the administered dose could not solve the problem. It was concluded that

dipyridamole is a very dose-sensitive compound to work with. The potentiation of the HABR and the response to infused adenosine reported before (Lautt et al., 1985) was obtained when the drug was administered intravenously. This suggests that dipyridamole can have effects other than blockade of adenosine uptake. It is noteworthy at this stage that responses to infused adenosine, the buffer capacity and autoregulation showed clear potentiation in all 8-PT studies, during the wearing off of the blocking property as well as after very low dose administration of the blocker (0.1 mg/Kg). It was difficult to maintain this potentiation response for long intervals. The only explanation I can suggest to explain this phenomenon is that 8-PT at low dose may have more potent affinity to block the adenosine uptake system than its affinity to the adenosine receptors. The suggested alternate methodology to study the potentiation of HABR and autoregulation is to replace dipyridamole by adenosine deaminase inhibitors. Despite the outcome of such a study, the available information supports strongly the washout hypothesis and that adenosine is the intrinsic dilator that controls the buffer response and autoregulation of the hepatic artery.

It has previously been concluded that the mechanism of the HABR and autoregulation is myogenic (Greenway and Stark, 1971; Hanson and Johnson, 1966; Hanson, 1973). The ability of papaverine, a potent direct smooth muscle relaxant, to abolish

both the hepatic arterial buffer capacity and the hepatic arterial autoregulation was considered as support for the myogenic hypothesis (Hanson, 1973). However, a potent vascular dilator like papaverine can mask the effect of locally produced vasodilators as well. Therefore, papaverine-induced inhibition of the buffer capacity and autoregulation cannot differentiate mechanisms. To confirm this interpretation we tested for autoregulation before and during infusion of isoproterenol (0.2 ug/Kg per min, intraportal). The isoproterenol resulted in dilation of the hepatic artery and eliminated the autoregulatory ability of the artery. As stated before, it is unclear in our study and in studies reported in the literature, why many animals do not show HABR or autoregulation, but a low basal vascular tone can certainly prevent the response and may account for some of these failures.

Administration of 8-PT or 8-SPT in blocking doses did not cause significant alteration of basal hepatic vascular parameters (Table 1) confirming previous results (Lautt and Legare, 1985). Dose-related vasoconstriction is induced by 8-PT but only after evidence of complete blockade of adenosine has occurred. If adenosine is crucially linked to regulation of the vascular tone in the hepatic artery, as we suggest, it is unclear how vascular tone can remain unaltered (despite some mild vasoconstriction) by low doses of 8-PT. However, most compounds of the xanthine family possess some degree of

anti-phosphodiesterase activity (Smellie, Davis et al., 1979). The inhibition of this enzyme prevents intracellular degradation of cyclic AMP and the resultant accumulation might lead to a dilator effect that counters the expected constrictor responses resulting from adenosine receptor blockade. Regardless of the explanation for the lack of effect of 8-PT on basal resistance, it is fortuitous since these and related vascular studies would be difficult or impossible to perform under a state of massive vasoconstriction or dilation. Since 8-PT caused mild constriction (no mean change) in some cats we felt it was important to determine if small amounts of vasoconstriction would significantly effect autoregulation. Norepinephrine infusion into the portal vein caused significant vasoconstriction but did not significantly alter autoregulation judged by several indices. This would suggest that altered basal hepatic nerve activity would also leave autoregulation unaltered. Torrance (1961) found that ganglion blockade by hexamethonium did not alter the shape of the pressure-flow curve but it did shift it somewhat up and to the left. Atropine did not alter the shape or position of the curve in his study. Most previous studies were reported in denervated livers but we specifically left the hepatic nerves intact.

4.6 METHODS OF QUANTITATING AUTOREGULATION

All methods of quantitation of autoregulation involve manipulation of data derived from a pressure-flow curve. Figure 18 shows a hypothetical curve similar to the curves obtained from individual or pooled experiments (Figures 15 and 17). The classical shape of a pressure-flow curve is sigmoid and concave to the pressure axis. At zero flow the curve intersects the pressure axis at the critical closing pressure (ccp), a pooled value averaging 11.5 $\pm$ 1.4 mmHg, which is slightly but significantly ($P<0.02$) higher than the portal venous pressure (8.5 $\pm$ 0.6 mmHg). A pressure-flow curve is not a form of data presentation that allows quantitation of autoregulation without further data manipulation. Quantitation of autoregulation can be done in several ways and, for demonstration of blockade of autoregulation, the index used must be carefully evaluated. The need to standardize the pressure ranges tested in the control and blocked state is the most obvious of the requirements. In addition, the autoregulatory index used must give a quantitative value that can be statistically compared in various states. While the change in the shape of the pressure-flow curve, as seen in Figures 15 and 17, is qualitatively convincing of reduced autoregulation, further data manipulation is required to allow useful statistical comparisons. The earliest studies used changes in calculated vascular resistance as a means of quantitation.

Parenthetically, we advocate the use of conductance (the inverse of resistance) as an index of arterial tone, as suggested by Stark (1968). An example of the distortion that resistance calculation imposes on data is seen where resistance is calculated to rise to infinity at low pressures. Conductance, on the other hand, decreases to zero, a value that can be mathematically used and that intuitively is more appealing. Nevertheless, the literature on autoregulation has exclusively used resistance calculations and we have conformed to that method for ease of comparison.

Calculation of resistance or conductance is problematic because it requires that assumptions be made about the appropriate pressure gradient across the resistance vessels. Virtually all of the early studies used arterial minus venous pressure and were required to report data for <u>total</u> vascular resistance. Ideally, the pressure gradient across the arterial resistance vessels should be used if the data are to reflect events at the arterial resistance site. The practical problem, however, is severe and depends on the fact that one cannot accurately and continuously measure capillary blood pressure. The use of critical closing pressure in some later reports and the introduction of the autoregulatory index and its common usage indicates some of the attempts to more realistically reflect events at the arterial resistance site. It is noteworthy that calculation of resistance using zero venous pressure was the only means that did not show clear

autoregulation in the control state. The use of either ccp to calculate the pressure gradient, for resistance changes or for the autoregulating index, showed the presence of autoregulation in the control state and its reduction after adenosine blockade.

The theoretical advantages and disadvantages of critical closing pressure use are of significance for most tissues and the logic for its use lies in the assumption that, if flow stops at a given arterial pressure then that arterial pressure is not contributing to a functional pressure gradient at any flow. However, the obvious objection is that the physical conditions that lead to complete collapse of the blood vessels at zero flow may have absolutely no relevance to vascular status during flow state. Fortunately, this problem is resolved in the liver because one can accurately and continuously measure sinusoidal pressure and can therefore accurately calculate the arterial pressure gradient. Recent reports (Lautt, Greenway et al., 1986; Greenway et al., 1985; Lautt et al., (in press)) indicate that, under basal conditions, including during complete occlusion of the hepatic artery, virtually all of the pressure drop from the portal vein to the inferior vena cava occurs across hepatic veins, that is, the sinusoidal pressure is insignificantly (0.2 - 0.6 mmHg) lower than portal venous pressure. All calculations of autoregulation have also been done using PVP as a correcting factor to calculate pressure gradient across the hepatic

arterial resistance site before the blood enters the sinusoids. It is interesting that the close correspondence of the ccp and PVP resulted in no significant differences in calculated resistance or changes in resistance regardless of which pressure was used. Clearly, however, the ideal situation of accurate estimation of arterial pressure gradient across the resistance site of the artery can be made and should, therefore, be used in all future calculations of hepatic arterial resistance.

Regardless of whether resistance is calculated using the appropriate pressure gradient, the means of quantitating autoregulation is still problematic. For demonstration of autoregulation in a basal state one can use either of the following two methods. Reduction of vascular resistance as blood pressure is decreased (Figure 19) or calculation of the autoregulatory index (Table 2) is useful for this purpose. In addition, these indices showed antagonism of autoregulation by adenosine receptor blockade. The autoregulatory index compares flows and pressures from a standardized control state to the shoulder of the pressure-flow curve and the calculated value is essentially a proportional change in flow divided by a proportional change in pressure. Of concern to us was the fact that comparison of control and blocked state required use of arbitrary, standardized pressure ranges to be used. In several animals we saw a clear upward shift of autoregulatory range when the blocking dose of 8-PT was too low (the curve

shifted to the right). In these cases the final slope of the absolute pressure-flow curve appeared similar, indicating that autoregulation may not have been altered at higher pressures but because the curve had shifted to the right, comparison with the control state would artifactually indicate blockade of autoregulation if the resistance changes over the standardized pressure range were compared. Others (Torrance, 1961) report shifts in the curve with curve shape being retained. To obviate this potential artifact we introduce a new index of autoregulation, the autoregulatory slope index. This calculation is based on comparison of the mean slope of the curve over the autoregulatory range divided by the mean slope below the autoregulatory range expressed as percent (but excluding any tailing-off of the curve near the critical closing pressure). This is illustrated in Figure 18. A slope index of 100% indicates a perfectly linear curve and does not depend on the position of the curve on the pressure axis or on other methods of standardizing the curve. The slope index in the blocked state is calculated in the same manner, identifying a new shoulder from the raw data. This approach is necessary since the blockers appeared to cause the shoulder to shift (but not consistently up or down) and by calculating the slope index independently in control and blocked state, bias can be eliminated. The slope index was significantly elevated by adenosine antagonists (Table 2). Because it avoids the need to calculate resistance, with the incumbent

errors in such calculation, this method appears useful. The usefullness may be even greater in studies of vascular beds where a valid value for capillary pressure cannot be made since the philosophical debate about the use of critical closing pressure to calculate pressure gradient is avoided. The principle disadvantage is that two linear slopes must be calculated from a non-linear curve and if this is not done mathematically, significant bias could be introduced. Several data points must, therefore, be obtained above and below the autoregulatory range. Regression calculation showed that the small non-linearity of the curves could be readily fitted to a straight line with correlation always greater than 0.95.

4.7 SUMMARY

The hepatic circulation is unique in that the liver receives a dual blood supply through the hepatic artery and portal vein. Under normal conditions the hepatic artery contributes 25% to total hepatic blood flows. The liver has no control over portal flow changes since portal flow is the sum of flows drained from prehepatic splanchnic organs. Therefore, the hepatic artery is the only means available for the liver to control its total blood flow. The smallest hepatic functional unit is the acinus which is a cluster of hepatocytes around a stalk of vascular structures and nerve fibers. These vascular structures include portal venules, hepatic arterioles, bile ductules and lymphatics. Blood flows

from the portal venules and hepatic arterioles into the hepatic sinusoids to be collected by the terminal hepatic venules. Flow in adjacent sinusoids is concurrent and under 3-5mmHg pressure gradient.

The existence of stable total hepatic blood flow is essential for the liver to maintain its clearance and capacitance (reservoir) functions as well as to maintain a stable intrahepatic pressure to keep sinusoids potent and to prevent their collapse. It was observed over seven decades ago that the hepatic arterial flow changes, in a reciprocal fashion, in response to changes in portal flow in order to maintain a stable total hepatic blood flow, a phenomenon termed recently "the hepatic arterial buffer response (HABR)". The ability of the hepatic artery to autoregulate its own resistance, regardless of portal flow, in response to changes in the hepatic arterial blood pressure was still controversial.

This study was designed first, to test and quantitate the hepatic arterial buffer response and the hepatic arterial autoregulation. Second, to explore whether one unified mechanism can explain both responses. An anesthetized splenectomized cat model with intact hepatic blood supply and nerve supply was used. The arterial flow and pressure in both the hepatic artery and the superior mesenteric artery were controlled mechanically through the application of a micrometer screw clamp around each artery. Flows were

measured by electromagnetic flow probes. Such a preparation allowed reproducible testing for HABR and autoregulation, however the range of testing could not be extended to higher hepatic arterial flow levels.

The buffer capacity of the hepatic artery in response to portal flow changes was found to be variable. A maximum value of 24% was reached at 60% decrease in portal flow. Further decreases in portal flow were less compensated for despite the progressive increased dilation of the hepatic artery. A constant buffer capacity of 30% was achieved, regardless of the level of portal flow changes, when pressure gradient across the hepatic artery was left uncontrolled. The hepatic artery is confirmed to be a weakly autoregulatory blood vessel as well.

Both the HABR and autoregulation can be blocked in a dose-related fashion by selective adenosine receptor antagonists. The blockade of autoregulation is roughly in parallel with blockade of the hepatic arterial buffer response and the response to infused adenosine. Theoretical aspects of quantitation of autoregulation led to the development of a new autoregulatory slope index that relies on the slope of the pressure-flow curve and makes no assumption about critical closing pressure, capillary pressure or resistance calculation. This new index confirmed adenosine involvement in hepatic arterial autoregulation. The data are consistent with the mechanism that an intrinsic regulator, namely

adenosine, mediates the HABR and hepatic arterial autoregulation. A unified theory of regulation of the hepatic artery is that adenosine concentration in the fluid that surrounds the hepatic arterial resistance vessels is regulated primarily by washout into blood streams. Washout into portal blood accounts for the hepatic arterial buffer response whereby reduced portal flow leads to less washout and the resultant accumulation of adenosine leads to arterial filtration; washout into the hepatic artery accounts for autoregulation whereby reduced arterial flow leads to reduced washout and the accumulation of adenosine leads to arterial dilation.

5. PROSPECTIVE STUDIES

The accomplishment of this research work left us with many unsolved questions. Quantitation of the HABR in the conscious cat was a project I started with. As mentioned in the text, I tried to measure blood flow to the liver using the microsphere technique. I learned the technique and applied it to conscious cats, however unexpected obstacles appeared. On many occasions the splenic artery, similar to the hepatic artery, dilated in response to reduced SMA flow. Therefore, simultaneous splenic and SMA occlusion are required to test for the HABR in conscious preparations. The behavior of the splenic artery raises many interesting questions about its role in splanchnic hemodynamics and whether there is any sort of relation between the hepatic artery and the splenic artery.

Direct measurement of adenosine concentrations in the fluid of the space of Mall is a clear target to prove or disprove the washout theory. The suggested methodology is to induce hepatic congestion (partial hepatic outflow occlusion) and to test for the presence of adenosine and its metabolites in the fluid exudate (which includes hepatic lymph) collected from the surface of the liver. If this technique can be validated to measure adenosine concentration in the space of Mall, many tests can be conducted to test for the rate of production, the ability of the portal vein and the hepatic artery to wash away adenosine, mechanism of release, etc.

The study of the pharmacokinetics of adenosine deaminase and deaminase blockers on hepatic circulation is essential. The HABR and autoregulation should be tested during their administration.

The most stable factor in our preparation is the portal pressure. It remains stable despite the wide variations in portal and hepatic arterial flow. There should be a mechanism to regulate the postsinusoidal resistance to maintain portal pressure constant. Such a mechanism is not adenosine mediated. Adenosine and other dilators seem to have no effect on portal pressure. The portal vein only responds to vasoconstrictors by increasing its resistance to flow (i.e. increased portal pressure). The exploration of this field is quite essential for the proper understanding of liver hemodynamics.

The speculation that two adenosine pools exist in the liver is interesting. The role of adenosine in some liver diseases, such as cirrhosis, is of interest to a person with a medical background, like myself. It is well known that some liver cirrhosis is accompanied by hyperdynamic circulation. Is it possible that cirrhotic livers are hypoxic and release increased amounts of adenosine which leads to the decreased peripheral resistance and the depressed cardiac contractility? Comparative studies to test for arterial adenosine levels in control and cirrhotic liver models are required. The effect of portal adenosine deaminase infusion on the systemic

circulation in such cirrhotic models is also required.

The microsphere technique allows the direct blood flow measurement in conscious preparations. Such a technique gives me the chance to test for the existance of postprandial intestinal hyperemia in cats and whether that hyperemia is adenosine mediated or not.

Despite the great advance in the understanding of the splanchnic hemodynamics and hepatic circulation in particular, this field can still be considered as a virgin land. Many questions are still waiting to be answered. Until a comprehensive understanding of the hepatic hemodynamics can be reached, many breakthroughs are still ahead to be discovered.

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APPENDIX A

Effect of vasoconstrictor and dilator on basal and autoregulatory parameters in the hepatic artery of the anesthetized cat.

	CONTROL	RESPONSE	P
NOREPINEPHRINE (N=5)			
basal HAC ($ml.min^{-1}.Kg^{-1}.mmHg^{-1}$)	0.156 ± 0.020	0.102 ± 0.015	**
CCP (mmHg)	17.8 ± 1.4	23.4 ± 1.9	**
% Δ HAR	-14.38 ± 4.39	-10.34 ± 5.60	NS
ARI	0.902 ± 0.088	1.046 ± 0.108	NS
Slope Index	50.45 ± 3.94	72.01 ± 11.71	NS
ISOPROTERENOL (N=4)			
basal HAC ($ml.min^{-1}.Kg^{-1}.mmHg^{-1}$)	0.189 ± 0.019	0.247 ± 0.025	*
CCP (mmHg)	25.3 ± 3.8	22.5 ± 2.4	NS
% Δ HAR	-16.29 ± 6.26	10.08 ± 8.93	*
ARI	1.072 ± 0.081	1.302 ± 0.068	NS
Slope Index	50.12 ± 9.33	80.52 ± 19.19	NS

The vasoconstrictor response produced by the intraportal infusion of norepinephrine ($0.1ug.min^{-1}.Kg^{-1}$) was accompanied by reduction in autoregulatory parameter values (% Δ HAR, ARI, and slope index), however, the decrease was not significant. Isoproterenol infusion ($0.2ug.min^{-1}.Kg^{-1}$) induced an opposite response (dilation) with significant inhibition of the hepatic arterial autoregulation according to one parameter (%ΔHAR).
* = $p<0.05$; ** = $p<0.01$; NS = not significant.

APPENDIX B

CONTROL DATA (Pooled from Co^{57} and Tin 113 Study)

	Right Kidney	Left Kidney	Lungs	Liver	Pancreas	Small Intestine	Large Intestine	Omentum	Stomach
Weight (gm)	9.12	9.45	16.74	76.00	5.72	68.07	20.93	65.30	23.30
	0.51	0.51	0.85	2.67	0.26	3.76	0.91	4.50	0.98
Weight (gm/Kg b.w)	3.47	3.60	6.34	28.97	2.17	25.81	7.98	24.55	8.82
	0.18	0.21	0.28	1.22	0.08	1.25	0.37	1.35	0.27
% of Cardiac Output	8.62	9.16	7.56	15.63*	0.41	15.58	4.07	1.51	0.96
	0.47	0.59	1.89	1.95	0.06	1.72	0.41	0.14	0.05
Flow (ml/Kg b.w)	10.28	11.24	8.59	18.75†	0.50	18.99	4.75	1.78	1.09
	1.09	1.45	1.28	2.98	0.08	2.32	0.62	0.20	0.09
Flow (ml/100g)	296.00	302.84	123.65	65.27	23.93	72.02	59.88	7.20	12.34
	27.92	30.34	17.83	9.01	4.40	7.68	7.58	1.01	1.11
Correlation Cofficient (r) of Co^{57} and Tin 113	0.957	0.865	0.973	0.850	-----	0.804	0.900	0.973	0.876
	0.026	0.048	0.012	0.032		0.039	0.034	0.017	0.041

* This data is just for HAF, Portal flow is 22.54 $\pm$ 2.20 ; Total Hepatic Blood Flow is 38.17 $\pm$ 3.59 %

† This data is for HAF, Portal flow is 27.07 $\pm$ 3.09 ; Total HBF is 45.82 $\pm$ 5.54

APPENDIX B

Control values (mean ± SE) pooled from 19 observations. The control values were calculated from the microsphere study conducted to evaluate the surgical preparation in anesthetized splenectomized cats (N=12). The average weight of cats was 2.64 ± 0.06 Kg. Two kinds of microspheres were used in this study ^{57}Co (10 observations) and ^{113}Sn (9 observations). Despite the ligation of the gastric, gastroduodenal and inferior mesenteric arteries, the splanchnic organs maintained an adequate blood supply. This indicates the presence of efficient anastamotic connections with the only remaining patent arterial flow, the superior mesenteric artery. The high percentage of cardiac output to the lungs indicated the existence of significant shunts from the arterial to the venous side, probably through the skin circulation. The significant correlation between ^{57}Co and ^{113}Sn activites in each tissue sample indicates symmetrical distribution of both kinds of microspheres, i.e. the first microspheres entrapment did not affect the pattern of distribution of the second ones.

APPENDIX C

Effect of SMA Occlusion on Systemic and Splanchnic Circulation in the Anesthetized Cat

	CONTROL	SMA OCCLUSION	
Portal Flow (ml/min)	75.28 ± 23.13	6.00 ± 1.51	
Portal Flow (%)	100	8.49 ± 1.36	
Pancreas	1.99 ± 0.21	6.60 ± 1.87	
Stomach	4.83 ± 0.71	37.96 ± 12.16	
S. Intestine	68.52 ± 1.49	11.63 ± 5.25	
L. Intestine & Rectum	17.96 ± 1.17	36.40 ± 6.79	
Omentum	6.69 ± 0.52	7.41 ± 2.00	
Cardiac Output ($ml.min^{-1}.Kg^{-1}$)	136.62 ± 28.50	128.23 ± 25.03	NS
Blood Pressure (mmHg)	111.4 ± 9.7	110.6 ± 12.0	NS
Pulse (beat/min)	226.2 ± 8.9	217.8 ± 7.9	*
HAF/THBF (%)	39.63 ± 2.40	93.09 ± 0.54	

The data present here are calculated from the microsphere study (N=5) where the first batch of microspheres was administered before SMA occlusion (control) and the second batch was administered after the occlusion (i.e. during the hepatic arterial buffer response (HABR)). Flowprobe measured portal flow (control) was 88.84 ± 27.87 ml/min. Complete occlusion of SMA left some residual portal flow according to the microsphere study, however, the correlation between net reduction in portal flow measured by the flowprobe and that calculated from the microsphere technique was highly significant ($r=0.998$; $P<0.0001$). The SMA occlusion did not affect the cardiac output or the systemic blood pressure, however, the pulse rate decreased significantly.
NS = not significant; * = $P<0.05$; THBF = total hepatic blood flow.

APPENDIX D

Control values (Mean ± SE) derived from the microsphere study conducted on conscious cats (N=6). In this study the splanchnic circulation was intact and cats were fasted overnight with free access to water. The average weight of cats was 2.90 ± 0.20 Kg. Two kinds of microspheres were used ^{57}Co (N=4) and ^{113}Sn (N=2). The cardiac output was 219.99 ± 32.55 $ml.min^{-1}.Kg^{-1}$. Portal flow was 25.89 ± 3.35% of the cardiac output, or 54.77 ± 7.44 $ml.min^{-1}.Kg^{-1}$. Total hepatic blood flow was 73.86 ± 12.97 $ml.min^{-1}.Kg^{-1}$. In conscious cats the hepatic artery contributed 23.04 ± 4.89% to total hepatic blood flow. This value is much less than that (39.63%) in unconscious splenectomized cats (Appendix C). Note that splenectomy deprives the hepatic circulation of 21.79% of portal inflow. The higher contribution of the hepatic artery to total hepatic blood flow in the unconscious splenectomized preparation is believed to be due to the partial stimulation of the HABR in response to reduced portal inflow due to splenectomy and surgical stress.

APPENDIX D

	Right Kidney	Left Kidney	Lungs	Liver	Pancreas	Spleen	Stomach	Small Intestine	Large Intestine & Rectum	Omentum
Weight	11.29	10.84	16.17	97.35	7.08	9.79	21.96	72.40	21.69	88.84
(gm)	0.94	0.95	0.52	4.72	0.64	0.91	1.12	6.24	1.37	14.55
Weight	3.94	3.78	5.71	34.24	2.50	3.39	7.71	25.61	7.60	29.55
(gm/Kg)	0.31	0.30	0.35	1.75	0.27	0.25	0.50	2.99	0.51	3.67
% of Cardiac	5.33	5.39	1.68	6.58*	2.01	5.55	2.26	9.45	4.17	2.46
Output	1.05	1.06	0.73	2.19	0.37	0.68	0.28	1.25	1.06	0.28
Flow	10.83	10.96	3.60	19.08*	4.04	11.79	5.11	20.65	8.06	5.12
($ml.min^{-1}.Kg^{-1}$)	1.67	1.77	1.58	6.63	0.41	1.97	1.09	3.62	1.38	0.56
Flow	269.04	286.90	63.00	50.40*	191.33	403.57	76.17	112.32	115.58	19.73
($ml.min^{-1}.100g^{-1}$)	29.61	32.38	28.42	15.35	24.00	92.90	18.81	28.20	16.37	3.71
% of Portal	----	----	----	----	7.65	21.79	8.82	36.48	15.36	9.89
Flow					0.58	1.76	0.86	1.97	2.23	1.34

* Data represent that of the hepatic artery only.